FIV Position Statement

Austin Pets Alive! aligns with the wealth of scientific data proving that cats with FIV (Feline Immunodeficiency Virus) are equally deserving and capable of life, rescue, and adoption with little to no emphasis placed upon their viral status.

Contagious Nature Fears

A multitude of respected, published studies document that risk of horizontal transmission in shelters, homes, or clinics is reduced to statistically insignificant in sterilized cats, even those who cohabitate with negative cats in multi-pet homes and in group housing in shelters.

- Transmission of FIV among cohabiting cats

Lifespan Fears

Similarly, a multitude of respected, published studies document that there is no statistical difference in lifespan between viral positive and viral negative cats.

- Naturally Acquired FIV infection…: Prevalence, Disease Association and Survival Analysis
- Long-term Impact on a Closed Household of Pet Cats of Natural Infection of FCV, Panleuk and FIV

Quality of Life Fears

Again, ample studies exist proving that viral positive cats in the typical pet home are most frequently asymptomatic for the duration of their lives. Gathered data on comorbidities include manageable conditions such as gingivitis.

- Contrasting Clinical Outcomes in Two Cohorts of Cats Naturally Infected with FIV
- Clinical Findings and Survival in Cats Naturally Infected with FIV

Armed with these facts:

- APA! will not place adoption restrictions on FIV positive cats. Viral status will be disclosed at the time of adoption and education provided to the adopter. Sterilized viral positive cats may be adopted into homes with viral negative cats. Sterilized viral positive cats may be adopted to indoor/outdoor or outdoor-only homes.
- APA! will not consider FIV a special need.
- APA! will provide the same resources and opportunities to viral positive cats that it would a viral negative cat, including equal medical care, equal opportunity for life, and equal opportunity for adoption.
- APA! will never consider death for a FIV positive cat based solely on this viral status.

As such, APA! has created the following resources and protocols to guide and educate staff, volunteers, medical professionals, adopters, animal welfare organizations, and the greater community:

- FIV FAQ
- FIV Terminology Policy

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Austin Pets Alive! has recently changed its protocol regarding FIV testing. The following information addresses the rationale behind this decision.

Historically, all cats and kittens in our care were tested for FIV/FeLV using an in-house ELISA test. However, research has shown that less than 2.5% of the North American feline population test positive for either FeLV or FIV. Considering the low prevalence of these diseases, we considered whether routine testing for all cats was the best use of our resources. Every month, thousands of dollars are spent on testing, and this does not account for the time and staffing it takes to perform these tests. In addition, the results of these tests are often misunderstood.

Further, the litany of research available on FIV conclusively shows the virus causes little to no impact on a cat's health, lifespan, or ability to live with a viral negative cat when neutered and in the absence of severe fighting.

As such, Austin Pets Alive! has stopped testing all cats and kittens for FIV so that our limited resources can be spent in more meaningful ways in the fight to save the most at-risk pets who would otherwise face needless shelter euthanasia. We continue to test all cats and kittens for FeLV. Cats and kittens in our care will be tested for FIV only if the test, used as a diagnostic tool, will enable us to provide better medical care in warranted medical situations.

We encourage our partners in private practice to offer FIV testing to recent adopters if warranted and as part of important routine care. If you or the adopter have concerns over a test result, please know that Austin Pets Alive! will always accept our pets back without condition and we remain available for questions or concerns.

Sincerely,

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Article

Naturally acquired feline immunodeficiency virus (FIV) infection in cats from western Canada: Prevalence, disease associations, and survival analysis

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Abstract — This retrospective study evaluated epidemiologic features and disease associations of feline immunodeficiency virus (FIV) infection in client owned cats from western Canada. Among 1205 cats that were tested 66 (5.5%) were positive for FIV antibody (FIV^+) with a higher prevalence in males than females. FIV^+ cats were older than the overall population. Epidemiologic features and disease associations were compared between 58 FIV^+, but feline leukemia virus negative ($FeLV^-$) cats and 58 age and sex matched FIV-negative (FIV^-), $FeLV^-$ cats. FIV positivity was associated with a history of bite wounds, increasing age, and male gender. Lethargy and oral diseases were significantly associated with FIV positivity. Although several FIV^+ cats were euthanized, the survival time of FIV^+ cats after diagnosis was not significantly different from that of FIV^- cats. In summary, FIV prevalence was low in cats from western Canada, clinical signs/diseases were mild, and lifespan was not different in FIV^+ cats.

Résumé — Infection naturelle par le virus de l'immunodéficience féline (VIF) chez les chats de l'Ouest canadien : prévalence, associations de maladies et analyse de survie. Cette étude rétrospective a évalué les caractéristiques épidémiologiques et les associations de maladies de l'infection par le virus de l'immunodéficience féline (VIF) chez des chats appartenant à des clients de l'Ouest canadien. Parmi 1205 chats qui ont été testés, 66 (5,5 %) étaient positifs pour l'anticorps du VIF (VIF^+) avec une prévalence supérieure chez les mâles par rapport aux femelles. Les chats VIF^+ étaient plus âgés que la population globale. Les caractéristiques épidémiologiques et les associations de maladies ont été comparées entre 58 chats VIF^+, mais qui étaient séronégatifs pour le virus de la leucémie féline ($FeLV^-$) et 58 chats séronégatifs pour le VIF (FIV^-) et le virus de la leucémie féline. La séropositivité pour le VIF était associée à de longs antécédents de morsures, à un âge grandissant et au sexe mâle. La léthargie et les maladies buccales étaient souvent associées à la séropositivité pour le VIF. Même si plusieurs chats VIF^+ ont été euthanasiés, le taux de survie des chats VIF^+ après le diagnostic n'était pas significativement différent de celui des chats VIF^-. En résumé, la prévalence du VIF est faible chez les chats de l'Ouest canadien, les signes cliniques et la maladie étaient légers et l'espérance de vie n'était pas différente chez les chats VIF^+.

(Traduit par Isabelle Vallières)

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Introduction

Feline immunodeficiency virus (FIV), a Lentivirus within the *Retroviridae* family, was first isolated in 1987 from a colony of group-housed cats with a high prevalence of opportunistic infections and degenerative diseases (1). Since then, various clinical diseases and syndromes have been associated with FIV infection in cats (2–4). Retrospective serosurveys suggest that FIV has been present in the domestic cat population at least since 1966 (5). This virus is now recognized worldwide as endemic in the domestic cat population (6). Typically, cats are tested for FIV antibody in a dual enzyme-linked immunosorbent assay (ELISA) that also detects feline leukemia virus (FeLV) antigen. Western blot, polymerase chain reaction (PCR), or virus isolation can be used to confirm FIV positivity (7,8). Seroprevalence of FIV among sick cats varies from 2.5% to 43.9%, which are higher rates than those of healthy cats (3,9–13). Multivariate analysis indicates that age, sex, health status, cat's lifestyle, and source of the cat (stray, relinquished, or feral) are significantly associated with risk of seropositivity (6,12).

The hallmark of experimental and natural FIV infection is progressive disruption of immune function by virus-induced depletion of CD4+ T lymphocytes in peripheral blood, leading to lowering of the CD4 to CD8 lymphocyte ratio as seen with

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HIV infection in people (14–16). Loss of CD4+ T lymphocytes impairs immune function resulting in increased susceptibility to opportunistic infectious and degenerative diseases. Naturally infected FIV-positive (FIV^+) cats exhibit clinical signs of fever, hemopoietic disorders, dermatitis, otitis, lymphadenopathy, stomatitis, gingivitis, neurological diseases, ocular diseases, weight loss, lethargy, anorexia, emaciation, vomition, cystitis, nephritis, diarrhea, abscesses, skin diseases, renal insufficiency/failure, hepatic disease, and upper respiratory tract infections (4,17–22). Clinical signs are most often a reflection of opportunistic infections, neoplasia and/or myelosuppression, as the disease progresses (22).

Few studies have compared the prevalence of clinical signs and diseases in FIV-infected and uninfected cats. Although a higher prevalence of FIV in sick cats suggests that FIV contributes to disease development, many veterinarians hold the opinion that FIV^+ cats are clinically indistinguishable from FIV-negative (FIV^-) cats. Further, FIV may produce only subtle manifestations of immune-dysregulation resulting in long periods of subclinical infections, and a fairly normal life span and quality of life (15). Studies from Australia have found equal prevalence of FIV in healthy and systemically ill cats (13), with no statistically significant association between FIV-positivity and the occurrence of anemia, mucosal inflammation and infection, neoplasia, lymphadenomegaly, pyrexia, or opportunistic infections (23). Another study reported that FIV infection did not adversely affect life expectancy in closed household pet cats (24). There are limited studies on FIV seroprevalence in Canada and no studies have compared the prevalence of various diseases/clinical entities in naturally infected FIV^+ and FIV^- cats from western Canada. The purpose of this retrospective study was to determine the seroprevalence of FIV in client-owned cats from western Canada, and to compare clinical signs, diseases, and survival times in FIV^+ and FIV^- cats.

Materials and methods

Sample population of cats

The test group for this retrospective study consisted of 1205 domestic cats, from the western Canadian provinces of Saskatchewan, Manitoba, and Alberta that were evaluated for FIV/FeLV infection at the Veterinary Teaching Hospital (VTH), Western College of Veterinary Medicine (WCVM), between January 1996 and December 2006, inclusive. Cats were tested for FIV/FeLV for 1 of the following reasons: to establish retrovirus status before introduction to a new household; to evaluate possible underlying infection; to evaluate potential exposure to these viruses after a known fight with another cat; to establish retrovirus status before vaccinating for FeLV. None of these cats had been vaccinated for FIV as the FIV vaccine was approved for use in 2005 in Canada. Cats that tested FeLV-positive ($FeLV^+$) were eliminated from the study of association between clinical variables and FIV status.

Fifty-eight cats were positive for FIV and negative for FeLV. Therefore, 58 FIV^-/FeLV-negative ($FeLV^-$), randomly selected, age and sex-matched uninfected cats were identified from the test group of cats which was stratified into groups based on age and sex to compare clinical signs, disease associations, and survival times with FIV^+ cats. No distinction was made between cats that were sexually intact or neutered. Health records of these 116 cats were reviewed to obtain history, signalment, clinical signs, clinicopathological diagnoses at the time of testing, lifestyle (indoor/outdoor activity), living status (alive or dead), and reason for death. Cat owners were surveyed to determine additional clinical problems; current status (living or dead); if dead, age at the time of death and reason for death.

Table 1. Prevalence of feline immunodeficiency virus (FIV) antibody in cats that were also tested for the presence of feline leukemia virus (FeLV) antigen

Group by age (y) and sex	Number tested	$FeLV^-$ FIV^+ (%)	$FeLV^+$ FIV^+ (%)
< 1 y			
Male	78	1 (1.3)	0 (0)
Female	70	0 (0)	0 (0)
Total	148	1 (0.7)	0 (0)
1 to 5 y			
Male	230	10 (4.3)	2 (0.9)
Female	133	7 (5.3)	1 (0.8)
Total	363	17 (4.7)	3 (0.8)
6 to 10 y			
Male	142	17 (12)	4 (2.8)
Female	91	1 (1)	0 (0)
Total	233	18 (7.7)	4 (1.7)
11 to 15 y			
Male	86	8 (9.3)	0 (0)
Female	70	1 (1.4)	0 (0)
Total	156	9 (5.8)	0 (0)
≥ 16 y			
Male	18	4 (22.2)	1 (5.6)
Female	27	0 (0)	0 (0)
Total	45	4 (8.9)	1 (2.4)
Unknown age			
Male	164	8 (4.9)	0 (0)
Female	96	1 (1)	0 (0)
Total	260	9 (3.5)	0 (0)
Male	718	48 (6.7)	7 (1.0)
Female	487	10 (2.1)	1 (0.2)
Total	1205	58 (4.8)	8 (0.7)

Testing protocol

All 1205 cats were tested for FeLV antigen and FIV antibody by the immunology laboratory of Prairie Diagnostic Services Inc., Saskatoon, using a commercially available ELISA kit (SNAP Combo FeLV antigen/FIV antibody; IDEXX laboratories, Maine, USA). The reported sensitivities of the assay for detection of FeLV and FIV are 98.6% and 98.2%, respectively; and the reported specificities are 98.2% and 100%, respectively (Dietz M, IDEXX Laboratories, personal communication, 2005).

Statistical analysis

Clinical data were stored in a database (Microsoft Access, 2003) and analyzed to determine the associations between clinical variables and FIV status using chi-squared analysis with Epi Info software (version 3.4.1). The strength of association between FIV infection and individual or combination of clinical variables was determined by calculating the crude

Table 2. Summary of results for 1205 serum samples tested for feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV) antigen

Sex and mean age	FeLV$^-$/FIV$^+$	FeLV$^+$/FIV$^+$	FeLV$^-$/FIV$^-$	FeLV$^+$/FIV$^-$	Total
Males *(n)*	48 (6.7%)	7 (1%)	635	28 (3.9%)	718
Mean age (y)	7.9	7.8	5.8	4.1	5.8
Females *(n)*	10 (2.1%)	1 (0.2%)	462	14 (2.9%)	487
Mean age (y)	4.3	1.8	6.3	5.0	6.3

Table 3. Clinical signs, disease conditions, and lifestyle of FIV$^+$ and FIV$^-$ cats

Clinical signs, diseases, and lifestyle	FIV$^+$ (n = 58)	FIV$^-$ (n = 58)	Odds ratio (95% CI)	P-value
Prior bite wounds	17	5	4.4 (1.37–14.98)	0.004[a]
Anorexia	13	14	0.91 (0.35–2.34)	0.83
Lethargy	13	5	3.06 (0.92–10.76)	0.04[a]
Weight loss	7	11	0.59 (0.19–1.81)	0.3
Gastrointestinal signs (vomition and diarrhea)	4	10	0.36 (0.0–1.35)	0.08
Fever	3	0	∞0.24	
Lymphadenopathy	2	2	1 (0.1–10.38)	1
Oral disease (stomatitis/gingivitis/ periodontal disease)	23	6	5.7 (1.94–17.52)	0.0006[a]
Ocular disease	11	4	3.16 (0.85–12.72)	0.053
Respiratory disease	10	11	0.89 (0.3–2.5)	0.8
upper respiratory tract infection	7	10	0.66 (0.2–2.08)	0.43
lower respiratory tract infection	3	1	3.11 (0.27–8.05)	0.31
Renal disease[b]	9	14	0.67 (0.25–1.76)	0.37
Endocrinopathies	7	4	1.85 (0.4–8.1)	0.34
diabetes mellitus	3	2	1.53 (0.2–13.68)	1
hyperthyroidism	4	2	2.07 (0.31–17.1)	0.68
Skin disease	6	5	1.22 (0.31–4.99)	0.75
Otitis externa	6	2	3.23 (0.55–24.34)	0.27
Neoplasia	2	4	0.48 (0.06–3.25)	0.68
Anemia	2	1	2.04 (0.14–5.9)	0.56
Cardiac disease	1	4	0.24 (0.01–2.37)	0.36
Lifestyle[c]				
outdoor	21	9	2.72 (0.7–10.41)	0.14
indoor	6	7	0.37 (0.08–1.69)	0.17

[a] $P < 0.05$ considered significant.
[b] Renal failure was diagnosed in 6 of 9 FIV$^+$ cats and 2 of 14 FIV$^-$ cats.
[c] Lifestyle information available for 27 FIV$^+$ and 16 FIV$^-$ cats.

odds ratios and their 95% confidence intervals (CI). Survival analysis was done to determine the effect of FIV status on cats' longevity irrespective of reasons for euthanasia or death using Kaplan-Meier product-limit method which compares the survival curves using both the Logrank test and the Gehan-Wilcoxon test using Graph Pad Prism (Ver. 5). The end point in the survival analysis was death/euthanasia. The data for 19 FIV$^+$ cats and 36 FIV$^-$ cats were excluded from the survival analysis as they were lost to follow-up. Data for cats that were still alive at the end of the study were censored in the survival analysis because survival beyond the censoring day was an unknown future event (25). Statistical significance was set at $P < 0.05$.

Results

FeLV/FIV ELISA results by age group and sex are shown in Table 1. Among 1205 cats, comprising 718 males and 487 females, 58 (5.5%; 95% CI: 4.2–6.8) were positive for FIV antibody but negative for FeLV antigen, 8 (0.7%; 95% CI: 0.2–1.2) were positive for both FIV and FeLV (Table 1) and 42 (3.5%; 95% CI: 2.5–4.5) were positive for FeLV antigen but negative for FIV antibody (Table 2). Prevalence of FIV infection in male cats (n = 55; 7.7%; 95% CI: 5.5–9.6) was greater than in female cats (n = 11; 2.3%; 95% CI: 0.1–3.6). Prevalence increased with age, with the greatest prevalence (8.9%) in the ≥ 16 y age group (Table 1). The mean age of male FIV$^+$ cats (7.9 y; range: 8 mo to 19 y) was greater than the population mean for males (mean 5.8 y; range 0 to 21 y). The mean age of female FIV$^+$ cats (4.3 y; range 2 to 11 y) was less than the population mean for females (6.3 y; range: 0 to 21 y) (Table 2). Clinical signs and diseases for the 58 FIV$^+$, FeLV$^-$ cats and the 58 FIV$^-$, FeLV$^-$ cats are summarized in Table 3. Bite wounds, lethargy, and oral diseases (stomatitis, gingivitis, and periodontal disease) were significantly associated with FIV seropositivity ($P < 0.05$). Although the proportion of FIV$^+$ cats with ocular disease (n = 11) and fever (n = 3) appeared higher, the associations were not statistically significant ($P > 0.05$). Inflammatory, endocrine, neoplastic and degenerative conditions were not significantly associated with FIV positivity.

Table 4. Summary of reasons for euthanasia and mode of death of FIV$^+$ ($n = 28$) and FIV$^-$ cats ($n = 12$).

Number of cats	Reasons for euthanasia
FIV$^+$ cats[a]	
9	FIV positivity
6	Gingivitis, periodontal disease, stomatitis
4	Renal failure
3	Vomition and diarrhea
3	Unknown
1	Abdominal abscess
1	Lymphosarcoma
1	Hit by a car
FIV$^-$ cats	
2	Clostridial colitis, clostridial enteritis
2	Lymphosarcoma
2	Renal failure, renal insufficiency
1	Unknown
1	Chronic urinary tract infection
1	Neoplasia
1	Chronic anorexia
1	Hepatic lipidosis
1	Abdominal mass

[a] Four FIV$^+$ cats died naturally, 1 of renal failure, 1 of diabetic complications, 1 of diabetes and congestive heart failure, and 1 of unknown cause.

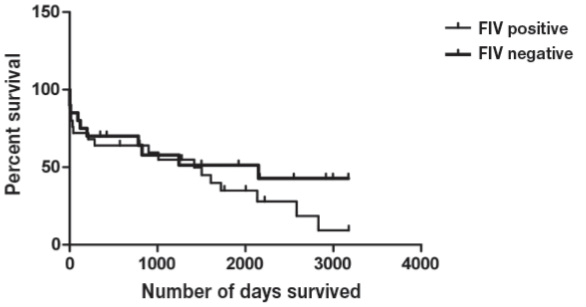

Figure 1. Kaplan-Meier survival curve comparison for FIV positive cats ($n = 39$) and FIV$^-$ cats ($n = 22$). The median survival times for FIV$^+$ and FIV$^-$ cats are 1416 and 2147 days, respectively. This difference is not statistically significant ($P > 0.05$). Ticks on the lines indicating censored items represent the data of cats that were alive at the end of the study.

Survival after FIV positive diagnosis

By the end of the study, 12 FIV$^-$ cats (euthanized, n = 12) and 32 FIV$^+$ cats (euthanized, n = 28) were dead. Among the 28 FIV$^+$ cats that were euthanized, 17 had been euthanized immediately after determining FIV seropositivity, and 3 cats had been euthanized within 30 d of diagnosis. The reasons for euthanasia/death and mode of death of FIV$^+$ and FIV$^-$ cats are summarized in Table 4. The mean age at the time of death for FIV$^+$ cats whose birthdates were available (n = 26) was 9.8 y (range: 8.6 mo to 17 y) while that for FIV$^-$ cats (n = 12) was 12.9 y (range: 7 to 21.5 y). The mean age of FIV$^+$ cats that were still alive (n = 7) at the end of the study was 13.2 y (range: 2.5 to 23.7 y) while that for FIV$^-$ cats (n = 10) was 9.6 y (range: 5 to 15.9 y). The clinical information available for 6 of 7 FIV$^+$ cats that were still alive by the end of the study is as follows: 4 cats were reported healthy although 1 was blind; 1 cat had renal failure and another cat had a heart murmur. The FIV status had no significant effect ($P > 0.05$) on the cats' longevity (Figure 1). The median survival times of FIV$^+$ (n = 39) and FIV$^-$ cats (n = 22) after FIV testing were 3.9 y and 5.9 y, respectively.

Discussion

This study examined seroprevalence of FIV infection in client-owned cats from western Canada and compared the prevalence of various clinical signs and disease entities as well as survival times in FIV$^+$ and FIV$^-$ cats. Several studies have reported a wide range of clinical signs associated with FIV infection; however, few studies have compared clinical signs and disease entities between age and sex matched FIV$^+$ and FIV$^-$ cats (17,23,24). Bias may have been introduced in the selection of cats to be tested for FeLV/FIV in this study as all cases were from a single teaching hospital and the criteria for retroviral testing may vary among clinicians. In addition, the prevalence of FIV may have been overestimated since most cats were presented with a clinical problem. Vaccination for FIV was not considered to be an explanation for a positive test result due to timing of the study relative to the release of FIV vaccine in Canada, lack of promotion of FIV vaccination at the WCVM VTH, and lack of history of FIV vaccination in individual medical records.

The reported sensitivity and specificity of the SNAP combo ELISA kit are high, but false positive and false negative test results can occur due to undetermined reasons. In a recent study, the performance of 7 FIV tests was compared, with the finding that the lowest sensitivity and specificity of the SNAP Combo test were 93.1% and 98.5%, respectively (26). Based on the reported test performance, the prevalence in the present study is likely overestimated and the true prevalence would be likely lower. In general, Western blot assay is recommended to confirm FIV ELISA results; however, confirmatory analyses were not performed given the retrospective nature of the study and the fact that the reported sensitivity and specificity for the SNAP combo ELISA kit are high.

The overall rate of FIV infection among 1205 cats tested in this study was 5.5%, which is comparable to prevalence studies for North America (2.5%) (12), Ontario, Canada (5.9%) (27), and Atlantic Canada (7.6%) (28). However, the prevalence was less than that reported in Germany (10%) (8), Australia (20.8%) (11), Turkey (22.3%) (29), Italy (24%) (10), and Japan (43.9%) (3). The findings of greater prevalence in male and adult cats (≥ 6 years old) are in agreement with previous studies (3,8,9,12,13,29).

In contrast to the general opinion that FIV infection significantly reduces a cat's life span, FIV status did not affect the cats' survival times significantly ($P > 0.05$) in this study despite the number of FIV$^+$ cats that were euthanized at or shortly after FIV diagnosis. Relatively high numbers of cats were excluded in the survival analysis because they were lost to follow-up after FIV testing. Their inclusion as censored items in the survival analysis did not affect the end result ($P > 0.05$) (data not shown). The results of survival time data analysis are in agreement with another study on the long-term impact of

FIV infection in a closed household of naturally infected pet cats (24). The fact that life span was not reduced with FIV infection may relate to a long incubation period for this virus after initial infection as cats may remain relatively disease-free for 8 y or more (30). Alternatively, the cats may have been infected with less virulent strains (such as, Subtype B) of FIV or may not have been exposed to infectious/opportunistic agents after acquiring FIV infection. There are 5 subtypes (A to E) of FIV isolates based on sequence diversity in variable regions (V3 to V5) (31–35) and FIV subtypes exhibit considerable geographic clustering. Subtype A is found in the western United States and Europe, subtype B is found in Japan and the central and eastern United States, while subtype C is found in California and British Columbia (31,35). Subtypes D and E have been reported from Japan (36) and Argentina (34), respectively. A study from Ontario, Canada identified A, B, and C FIV subtypes with subtype A being the most frequent (37). Despite the heterogeneous geographic distribution, most FIV isolates belong to either subtype A or B. Studies have hypothesized that subtype B is in a more advanced state of host adaptation and may, therefore, be less pathogenic than subtype A (31,35). An observational and comparative study of specific pathogen-free cats experimentally infected with FIV subtypes A and B, demonstrated that a cat infected with subtype A virus developed progressive immunological abnormalities and severe clinical signs of immunodeficiency syndrome 8 y and 8 mo after infection, while cats infected with subtype B did not show any significant clinical signs of immunodeficiency syndrome during the same time period (30). No studies have been done to investigate the prevalence of various FIV strains in the western Canadian provinces comprising this study. This warrants further investigation.

FIV^+ cats in this study were significantly more likely to have had a history of bite wounds than were FIV^- cats. This virus is effectively transmitted through bite wounds and greater frequency of FIV infection in male cats may relate to fighting, territorial aggressiveness, and courtship fighting (9). The sample population herein was not segregated into neutered and entire animals. Although reports vary, there is evidence that neutering and spaying do not have a statistically significant effect on FIV prevalence (28,29). In addition, although neutered cats do not indulge in courtship fighting, they still retain territorial aggressiveness (29).

$FeLV^+$ cats were eliminated from the study of FIV and its clinical association as this virus has a severe influence on disease progression and life expectancy of FIV co-infected cats (16,38). Opportunistic infections by viruses, bacteria, protozoa and fungi have been reported in experimentally and naturally infected FIV^+ cats (3,39,40). There were no serious/life-threatening infections associated with FIV^+ cats in this study. The clinical course of FIV infection and pattern of opportunistic infections may vary with the patient and its lifestyle, geographic location, and strain of virus. Unlike previous reports, we found significant associations only with lethargy and oral disease in FIV^+ cats when compared with FIV^- cats. Although, anemia, hyperthyroidism, lower respiratory tract infection, renal failure, and outdoor lifestyle showed trends towards an association with FIV positivity (odds ratio > 2), the findings were not statistically significant ($P > 0.05$). To our knowledge no studies have compared clinical findings in FIV^+ cats with age and sex matched randomly selected FIV^- control cats. It has been reported that there is no significant disease association with naturally acquired FIV infection in Australian cats compared with uninfected cats (23), and that cats kept under hygienic conditions or infected by less pathogenic FIV strain(s) do not develop severe clinical signs (16).

Oral disease was significantly more common in FIV^+ cats than in FIV^- cats in this study. This agrees with earlier reports, and suggests that a systemic immunosuppressive effect of FIV may allow microbial invasion of the oral cavity (9,18,41). In this study, it was uncertain whether stomatitis was predisposed by other factors (dental disease, other viruses), as no additional diagnostic tests were done. Earlier reports suggest that increased ocular disease in FIV^+ cats could be due to direct damage from the virus or opportunistic infections (39,42,43) but ocular diseases were not significantly associated with FIV positivity in this study; despite a trend to increased numbers.

Lymph node enlargement is one of the most frequently reported abnormalities in cats with FIV infection. In our study, there appeared to be no association between FIV-positivity and lymph node enlargement, which is in agreement with the findings of Hosie et al (17) and Shaw et al (23). Further, there was no association between neoplasia and FIV infection in our study which agrees with the findings of Shaw et al (23) who analyzed the association of FIV infection with diseases in Australian cats. This is in contrast to Hopper et al (44) who determined that the prevalence of neoplasia in an FIV^+ cat population was increased.

In summary, FIV status did not significantly affect cats' longevity in this study. History of bite wounds, male sex, increasing age, lethargy, and oral diseases were associated with FIV positivity when compared with age- and sex-matched randomly selected FIV^- control cats. A significant proportion of cats that tested FIV positive were euthanized, perhaps prematurely. There may be geographical/strain-related variation in clinical signs, diseases, and opportunistic infections associated with FIV infection.

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Long-term impact on a closed household of pet cats of natural infection with feline coronavirus, feline leukaemia virus and feline immunodeficiency virus

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A closed household of 26 cats in which feline coronavirus (FCoV), feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) were endemic was observed for 10 years. Each cat was seropositive for FCoV on at least one occasion and the infection was maintained by reinfection. After 10 years, three of six surviving cats were still seropositive. Only one cat, which was also infected with FIV, developed feline infectious peritonitis (FIP). Rising anti-FCoV antibody titres did not indicate that the cat would develop FIP. The FeLV infection was self-limiting because all seven of the initially viraemic cats died within five years and the remainder were immune. However, FeLV had the greatest impact on mortality. Nine cats were initially FIV-positive and six more cats became infected during the course of the study, without evidence of having been bitten. The FIV infection did not adversely affect the cats' life expectancy.

THERE have been many publications describing the effects on cats of infections with either feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV) or feline coronavirus (FCoV) individually, and cats have been infected with combinations of these viruses in laboratory settings. However, this is the first report of a group of cats naturally infected with all three viruses and studied over a long period.

The rate of infectivity of FIV is extremely variable with reports in the literature stating that from 0 to 100 per cent (Table 1) of cats in contact with an FIV-infected cat may become infected. In addition, it has been recognised that FIV-infected cats kept under specific pathogen-free (SPF) conditions can survive for long periods after being first diagnosed (Kohmoto and others 1998). However, it is not clear whether this phenomenon is related solely to cats kept in an artificially disease-free environment or whether it also applies to cats in the field. Both FIV and FeLV are immunosuppressive agents, but there is little information about their ability to influence each other's infectivity. In households where FCoV is endemic, 5 to 15 per cent of infected cats may develop feline infectious peritonitis (FIP), and cats which are immunocompromised by a simultaneous retrovirus infection are believed to be at greater risk (Poland and others 1996).

This paper describes a study of the transmission rates of FIV, FeLV and FCoV among 26 cats in a closed household over a period of 10 years. The viral status of the animals and their survival from first diagnosis were monitored, and their causes of death were established specifically to determine whether the viruses might have been implicated.

MATERIALS AND METHODS

Husbandry

Twenty-six pet cats kept in one household were monitored for 10 years. The cats were allowed to mix with each other but none was allowed to roam outside. Only one cat (cat Z) was introduced during the 10 years; it was the only pedigree cat, a Persian, and had been rescued in May 1989 from its previous owner who wished it to be euthanased because it had an anti-FCoV immunofluorescent antibody titre of 1280. None of the cats was vaccinated against FeLV, feline panleucopenia virus, feline calicivirus or feline herpesvirus. Virological testing was begun in March 1988, because three cats had died during 1987 after developing anaemia, diarrhoea and suspected liver failure. *Haemobartonella felis* had been identified in one of the three cats.

The cats were blood tested annually; the blood samples were taken and any postmortem examinations were performed by one of the authors (J. D.). The blood samples and selected formalin-fixed tissue samples were examined serologically and histologically at the Feline Virus Unit, University of Glasgow.

Serology and virology

The samples were initially screened for FIV antibodies by using a commercial ELISA (FIV Petcheck; Idexx) and positive results were confirmed by Western blotting (Hosie and Jarrett 1990) or by immunofluorescence (IF) (Pedersen and others 1987). Samples examined after 1991 were tested by IF. FeLV antigen was detected by using an ELISA to detect p27 (FeLV Petcheck; Idexx, or Innochem; C. Lutz) and positive results were confirmed by virus isolation (Jarrett and Ganière 1996). Virus neutralising antibody titres to FeLV were measured as described by Jarrett and Ganière (1996). Antibodies to FCoV were measured by IF (Addie and Jarrett 1992), and faeces and saliva were monitored for FCoV by the detection of FCoV RNA by reverse transcriptase-polymerase chain reaction (RT-PCR) by the method described by Herrewegh and others (1995).

Pathology

Postmortem examinations were carried out on 15 of the 19 cats that died. Sections of formalin-fixed, paraffin-embedded tissue were cut at 5 µm and stained with haematoxylin and eosin. Selected sections were also stained with Giemsa stain. To investigate the phenotype of lymphoid tumours, paraffin-embedded sections were immunostained with anti-CD79a (mb-1), anti-CD3 and MAC 387 (anti-macrophage/anti-neutrophil) antibodies by the methods of Callanan and others (1996).

Statistical analysis

To compare the survival of the cats in the different groups, classified according to their viral infection status, Kaplan-Meier product-limit survival curves were generated. The Tarone-Ware statistic was used to assess whether any observed differences were statistically significant at the 5 per cent level. Where necessary, a Cox proportional hazard model was used to assess the hazard function associated with viral status, taking into account age at testing and seroconversion; again

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significance was set at the 5 per cent level. The longevity of the cats was also compared by using the Kaplan-Meier product-limit method.

RESULTS

Table 2 shows the sex and age of the cats at the end of study or at death, and the causes of death. Tables 3, 4 and 5 give the serological data for FCoV, FeLV and FIV, respectively.

FCoV antibody titres and FIP

Each cat had anti-FCoV antibodies on at least one occasion during the study (Table 3). The percentage of seropositive cats decreased from 92 per cent in July 1988 to 39 per cent in September 1989, but by March 1990, 94 per cent of the cats were again seropositive, indicating that they had been reinfected. There were two further similar cycles; the prevalence of seropositive cats decreased to 50 per cent by October 1993, increased to 88 per cent in December 1995, and decreased to 50 per cent in 1998.

The presence of FCoV-RNA in the faeces was monitored only once, in January 1996, when six of the remaining eight cats were shedding the virus. There was no absolute correlation between seropositivity and virus shedding, but all but one of the virus shedders had high antibody levels and the only seronegative cat, T, did not shed the virus.

Only cat B, which was also infected with FIV, developed FIP.

Transmission of FeLV

At the beginning of the study, seven of the cats were infected with FeLV and no more became viraemic. Cat X had initially been discordant in March 1988, was FeLV-negative in October 1988, but became persistently viraemic in March 1990. The titres of FeLV neutralising antibody were measured in 23 cats (Table 4). Four of the five FeLV-positive cats had no detectable virus neutralising antibodies and the fifth had an antibody titre of only 4. In contrast, 14 of the remaining 18 FeLV-negative cats had antibody titres of over 32 and only one was seronegative. None of these cats became FeLV-positive. Eight years after the first test and four years after the last FeLV-positive cat died, the virus neutralising antibody titres of four of the six remaining cats tested had decreased.

Of the four cats that were FeLV-positive but not also infected with FIV, cat X developed myeloid leukaemia and died at 10 years of age, 18 months after FeLV had first been isolated. Cats U and Y were euthanased at the start of the survey, within one and two months of being diagnosed, when they were six and nine years old, respectively. Cat U had an osteosarcoma involving its mandible. Cat Y was not examined postmortem. Cat M was euthanased six months after it was first diagnosed after it had developed acute respiratory distress and pallor; it was not examined postmortem. Within five years of being diagnosed, all the cats with FeLV infection had died.

Three of the seven FeLV-infected cats were also infected with FIV. Of these, cats H, R and W survived six, 24 and 58 months, respectively, after being diagnosed. Cat H was euthanased owing to intractable melaena six months after

TABLE 1: Previous studies of the transmission of feline immunodeficiency virus (FIV)

Number of cats infected	Number of cats in contact	Number of in-contact cats FIV-infected	Number of households	Reference
N	14	0	1	Yamamoto and others (1988)
16	31	0	16	Shelton and others (1989)
4	68	0	1	Shelton and others (1990)
N	34	1	N	Sparger and others (1989)
9	7	2	1	Pedersen and others (1987)
N	N	3-67%	N	Hosie and others (1989a)
N	27	14	N	Hopper and others (1989)
N	11	11	1	Hosie and others (1989b)

N Not given

TABLE 2: Sex, age at the end of the study or death, and the cause of death of the cats

Cat	Sex	Date of birth	Date of death	Age at death (years)	Age in Oct '98 (years)	†Survival (months)	FeLV/FIV status	Cause of death
A	MN	May 1981	Apr 1996	15		30	FIV	T cell alimentary lymphosarcoma*
B	MN	Aug 1983	June 1991	8		32	FIV	Feline infectious peritonitis*
C	MN	May 1982	Feb 1991	9		17	FIV	Thymoma*
D	MN	May 1981	July 1993	12		51	FIV	Chronic proliferative cholangitis*
E	MN	May 1987	June 1998	11		117	Neg	Chronic heart failure, alimentary carcinoma*
F	MN	pre 1975	June 1989	>14		8	Neg	Squamous cell carcinoma*
G	MN	June 1987	Nov 1993	6		61	Neg	B cell lymphosarcoma on face*
H	FN	June 1987	Apr 1989	2		6	FeLV, FIV	E; melaena
I	FN	1981	June 1989	8		8	Neg	Cardiomyopathy*
J	FN	1986	Jan 1991	5		27	Neg	Nephritis, cholangitis*
K	MN	Aug 1983	Nov 1992	9		49	FIV	E; heart failure, myocarditis,* glomerulonephritis, chronic bronchitis
L	FN	Apr 1980	Mar 1989	9		5	Neg	E; pale and respiratory distress
M	FN	Mar 1987	Apr 1989	2		6	FeLV	E; pale and respiratory distress
N	FN	Apr 1987			11·5	84	FIV	
O	MN	Apr 1987			11·5	48	FIV	
P	MN	Oct 1986			12	120	Neg	
Q	MN	May 1982	Nov 1989	7		13	FIV	Chronic interstitial nephritis*
R	MN	July 1982	Oct 1990	8		24	FeLV, FIV	Acute blast cell leukaemia*
S	MN	Feb 1984			14-5	84	FIV	Adenocarcinoma, glomerulonephropathy*
T	FN	Apr 1983			15-5	120	FIV	
U	MN	May 1982	Sep 1988	6		NA	FeLV	Osteosarcoma of jaw
V	FN	Sep 1981	Nov 1994	13		73	FIV	Bronchitis, cholangitis, *Haemobartonella felis**
W	MN	Apr 1983	Aug 1993	10		58	FeLV, FIV	E; intractable gingivitis, pharyngitis. Histology revealed focal interstitial nephritis, cholangitis, nodular hyperplasia of pancreas*
X	FN	May 1981	Sep 1991	10		‡18	FeLV	Myeloid leukaemia*
Y	F	1979	Mar 1988	9		NA	FeLV	E; poor body condition
Z	FN	Aug 1988			10	84	FIV	

* Histopathological diagnosis, † Survival in months since first FeLV and FIV tests, ‡ FeLV discordant in Feb 1988, negative Oct 1988 and 1989, and became FeLV-positive in March 1990
MN Male neutered, FN Female neutered, E Euthanased, NA Not applicable, diagnosed FeLV-positive at time of illness

TABLE 3: Antibody titres to feline coronavirus in the 26 cats during the study

Cat	Mar '88	Jul '88	Oct '88	Apr '89	Sep '89	Mar '90	Sep '90	Oct '91	Oct '92	Oct '93	Oct '94	Dec* '95	Oct '96	Oct '97	Oct '98
A		40	20	80	160	640	320	40	80	40	>1280	160+	Dead		
B		40	20	80	80	320	320		FIP June 91						
C		160	40	40	0	0	0	80	Dead						
D		80	40	20	0	640	640	80	0	Dead					
E		80	0	0	0	40	40	0	0	0	20	160+	160	160	Dead
F		40	0	0	Dead										
G		160	80	80	0	640	640	80	40	0	Dead				
H		160	160	80	Dead										
I		20	0	40	Dead										
J	160	10	0	0	0	320	640	Dead							
K		20	20	0	10	160	20	10	40	Dead					
L		80	20	Dead											
M	80	0	0	20	Dead										
N		160	80	160	1280	1280	1280	80	160	20	320	20+	320	80	0
O		80	80	40	320	640	1280	320	640	640	>1280	320+	640	1280	320
P		160	160	80	640		640	80	160	160	20	320+	320	160	0
Q		10	80	20	0	Dead									
R		20	0	10	20	40	0	Dead							
S		20	40	20	0	80	160	20	0	0	0	20–	0	10	80
T		0	0	0	0	80	0	0	0	0	0	0–		0	0
U		160	Dead												
V		20	10	0	0	40	160	10	0	0	0	Dead			
W		20	0	0	0	80	20	10	0	Dead					
X		80	40	10	0	320	160	Dead							
Y	160	Dead													
Z	[Acquired May '89 with IFA titre 1280]				20	160	1280	80	160	640	>1280	320+	320	320	320
SN		2	8	7	11	1	3	2	6	5	3	1	1	1	3
SP		23	15	15	7	16	15	12	7	5	6	7	5	6	3
%SP		92	65	70	39	94	83	71	54	50	66	88	83	86	50

* Virus shedding status, only cat A was shedding virus in saliva and faeces, the rest were shedding in the faeces only
SN Seronegative, IFA Immunofluorescent antibody, SP Seropositive, FIP Feline infectious peritonitis

diagnosis when it was two years of age (no histopathology available). Cat R developed acute blast cell leukaemia at eight years of age, 24 months after FeLV and FIV were first diagnosed. Cat W was euthanased 58 months after FeLV and FIV were diagnosed when it was 10 years old owing to untreatable chronic gingivitis and pharyngitis; postmortem examination revealed focal interstitial nephritis, cholangitis and nodular hyperplasia of the pancreas.

Transmission of FIV despite lack of aggression

The cats were first screened serologically for FIV in October 1988. Eight of the remaining 25 cats (cat Y, an FeLV-infected cat had died) were FIV-positive, and cat C was seropositive by ELISA but seronegative by IF (Table 3). Cat C and six more cats became seropositive over the next six years. According to the owners, the cats displayed little aggression, with the exception of occasional paw-flailing involving cats D, E and W; cat W

TABLE 4: Isolation of feline leukaemia virus (FeLV) and results of tests for virus neutralising antibody (VNA)

				FeLV status										FeLV-VNA titres		
Cat	Mar '88	Jul '88	Oct '88	Mar '90	Sep '90	Oct '91	Oct '92	Oct '93	Oct '94	Dec '95	Oct '96	Oct '97	Oct '98	Apr '89	Oct '91	Oct '97
A			–	–	–	–	–	–	–	–	Dead			>32	>32	
B			–	–	–	Dead								16		
C			–	–	–	Dead								>32		
D			–	–	–	–	–	Dead						>32	>32	
E		–	–	–	–	–	–	–	–	–	–	–	Dead	8	0	0
F			–	Dead										0		
G			–	–	–	–	–	–	Dead					>32	>32	
H			+	Dead										0		
I			–	Dead										>32		
J	–	–	–	–	–	Dead								>32		
K			–	–	–	–	–	Dead						>32	>32	
L			–	Dead												
M			+	Dead										0		
N			–	–	–	–	–	–	–	–	–	–	–	>32	>32	4
O			–	–	–	–	–	–	–	–	–	–	–	>32	>32	>32
P			–	–	–	–	–	–	–	–	–	–	–	>32	>32	8
Q			–	Dead										>32		
R			+	+	+	Dead								0		
S			–	–	–	–	–	–	–	–	–	–	–	16		8
T			–	–	–	–	–	–	–	–	ND	–	–	>32	>32	>32
U		+	Dead													
V			–	–	–	–	–	–	–	Dead				>32		
W			+	+	+		+	Dead						0		
X	+/–		–	+*	+	Dead								4		
Y	+	Dead														
Z	[Acquired May '89]			–	–	–	–	–	–	–	–	–			>32	

* Indicates first FIV seroconversion
ND Not done

TABLE 5: Results of tests for feline immunodeficiency virus (FIV) and period of survival from first diagnosis of FIV

Cat	Jul '88	Oct '88	Apr '89	Sep '89	Oct '91	Oct '92	Oct '93	Oct '94	Dec '95	Oct '96	Oct '97	Oct '98	Survival (months)
H		+	+	Dead									6*
Q		+	+	+	Dead								13
C		+/–†	–	+	Dead								17
R		+	+	+	Dead								24*
A		–	–	–	–	–	+‡	+	+	Dead			30
B		+	+	+	Dead								32
O		–	–	–	–	–	–	+‡	+	+	+	+	48
K		+	+	+	+	+	Dead						49
D		–	+‡	+	+	+	Dead						51
W		+	+	+	+	+	Dead						58*
V		+	+	+	+	+	+	+	Dead				73
N		–	–	–	+‡	+	+	+	+	+	+		84
S		–	–	–	+‡	+	+	+	+	+	+	+	84
Z	Acquired May '89			–	+‡	+	+	+	+	+	+	+	84
T		+	+	+	+	+	+	+	+	+	+	+	120
Y	Dead												
U	–	Dead											
F		–	Dead										
L		–	Dead										
M		–	–	Dead									
I		–	–	Dead									
J	–	–	–	–	Dead								
X		–	–	–	Dead								
G		–	–	–	–	–	–	Dead					
E	–	–	–	–	–	–	–	–	–	–	–	Dead	
P	–	–	–	–	–	–	–	–	–	–	–	–	

* Coinfected with FeLV, † Seropositive by ELISA but seronegative by IF, ‡ Indicates first FIV seroconversion

was infected from the start of the study, cat D became infected in its first year and cat E remained uninfected. There was no evidence of the cats biting one another.

Longevity of FIV or FeLV-infected cats after diagnosis

Seven of the 12 FIV (but not FeLV)-infected cats died at 13, 17, 30, 32, 49, 51 and 73 months after being diagnosed. The remaining five FIV-infected cats remained healthy to the end of the study, 48, 84 (three cats) and 120 months after being diagnosed. A comparison of the seronegative cats with the FIV-positive cats did not reveal any significant difference in longevity or survival from their first testing positive. There was a trend for the FIV-positive cats to survive longer in that their median survival time was 51 months compared with 17·5 months for the seronegative cats, but this difference was not statistically significant (Fig 1). The impact of FeLV was much more pronounced and the FeLV-positive cats, including those with concurrent FIV infections, survived for a significantly shorter time than the FIV-infected and seronegative cats ($P<0·01$); the median survival times of the FeLV-infected, FIV-infected and FeLV/FIV coinfected cats were six, 51 and 17·5 months, respectively. In no case was the cat's age when tested a significant covariate. FeLV infection also had a significant effect ($P<0·05$) on the cats' longevity, with median lifespan figures of 86·5 months for the FeLV/FIV coinfected cats, 150 months for the FIV-infected cats and 103 months for the retrovirus-negative cats.

Tissues from six of the seven FIV-infected cats showed a wide range of pathological changes. Cat A had an alimentary T cell lymphosarcoma and cat C had a thymoma. Other histopathological changes observed in individual cats included cholangitis, bronchitis, chronic interstitial nephritis, myocarditis, glomerulonephritis and *Haemobartonella felis*-associated haemolytic anaemia. As indicated above, cat B, which was FIV-positive and also had antibodies to FCoV, developed non-effusive FIP.

Throughout the study, seven of the 26 cats remained free of FIV and FeLV infection, but six of them died during the study. Three cats developed tumours: cat E was euthanased with severe ascites and dyspnoea, and histopathology revealed chronic heart failure and a carcinoma in the intestines. Cat F had a squamous cell carcinoma in its mouth, and cat G had a lymphosarcoma involving the buccal mucosa of its right cheek. These animals died at 11, 14 and six years of age, respectively. Cat I developed dilated cardiomyopathy, and cat J developed cholangitis and nephritis. Cat L was euthanased owing to acute respiratory stress in association with pallor, but it was not examined postmortem.

DISCUSSION

As far as the authors are aware, this is only the second longitudinal study of a household of cats naturally infected with FCoV, FeLV and FIV, a household of 73 cats having been monitored in retrospect between 1977 and 1980 (Shelton and others 1990). In the present study, the 10-year period of observation has made it possible to obtain a more definitive assessment of the relative impact of each of these persistent viral infections on the population. The pattern of transmission of FCoV and FeLV, and the diseases they produced, illustrated several important features about the diagnosis and epidemiology of the viruses, and was consistent with previous reports. The most remarkable observation was that FIV was transmitted fairly regularly throughout the period, in the absence of overt aggression between the cats, and that it had little effect on the survival of the cats.

FCoV had a modest effect on life expectancy, causing one case of FIP, although, as expected, because the virus is very

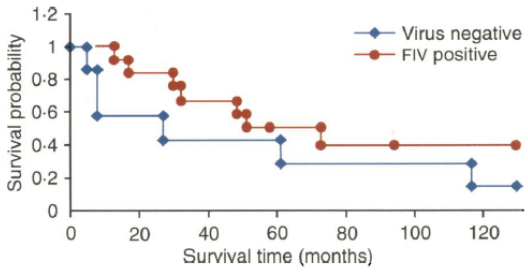

FIG 1: Survival curves of cats infected with feline immunodeficiency virus (FIV) and FIV-seronegative cats

contagious, all of the cats had at one time been infected. All but two of the cats were seropositive in the first full test in July 1988, and again in March 1990, after presumed reinfection. Since most of the cats in the household were FCoV-seropositive most of the time, it would not have been necessary to test all the cats to establish whether FCoV infection was present and a random sampling of, say, six cats would have been sufficient. However, this is only true because all the cats were mixing and were reinfecting each other; had the cats been separated into groups, FCoV would have died out in some groups and it would still have been necessary to test all the cats.

The anti-FCoV antibody titres of the cats decreased twice, in 1989 and again in 1992, indicating that in a closed household, that is, one which admits no new cats or kittens and in which the cats do not go outdoors, FCoV infection may eventually die out spontaneously. It is possible that by segregating the seropositive and seronegative cats FCoV infection could have been eliminated completely, as described by Gonon and others (1995). The percentage of seropositive cats decreased from 92 per cent in July 1988 to 39 per cent in September 1989. However, by March 1990, 94 per cent of the cats were again seropositive, indicating that they had been reinfected. The two possible sources of reinfection were the introduction of the seropositive cat Z in May 1989, or virus shed by one or more of the remaining seropositive cats. It seems unlikely that cat Z was the source of reinfection, because more cats became seronegative between the tests in April and September 1989, although cat Z was introduced in May. It is therefore more probable that the household was reinfected from within by, for example, cat O which is likely to have been a chronic FCoV shedder, because it was at no time seronegative. Alternatively, the persistence of FCoV in this household could have been due, at least in part, to the coinfection of many cats with FIV, because FIV-positive cats have been reported to shed 10 to 100 times more FCoV than FIV-negative cats (Poland and others 1996). However, the two cats which were not shedding FCoV in December 1995 were also FIV-positive. The FCoV antibody titres of cats which are not shedding the virus themselves, but are living with cats which are, can decline. However, within a few weeks, animals which have become seronegative usually begin shedding the virus again and seroconvert (D. D. Addie, unpublished observations).

Only one cat, cat B, died of FIP, 35 months after being found to be seropositive. This finding illustrates the fact that cats can be infected with FCoV quite unbeknownst to their owners because they may show no clinical signs and take a very long time before developing FIP. However, an incubation period of 35 months is very unusual because cats are most likely to die of FIP within six to 18 months of being infected (Addie and others 1995). The cat which developed FIP was also infected with FIV, so that its immunity may have been compromised by FIV, allowing FIP to develop. This explanation seems probable in view of the report of an experimental coinfection of cats with FCoV and FIV, in which two of 19 cats developed FIP within 10 weeks of becoming infected by FCoV (Poland and others 1996). That none of the FeLV-infected cats developed FIP is slightly surprising because FIP has been reported to be the third most common cause of death in FeLV-infected cats (Reinacher and Theilen 1987).

Cats A, B, K, N, O, P and Z sustained a four-fold or greater increase in antibody titre after the first reinfection, but only cat B developed FIP, confirming that a significant rise in antibody titre is not necessarily prognostic of the development of FIP. In experimental infections, seropositive cats develop FIP more rapidly than seronegative cats when they are reinfected. However, in natural infections, cats which have previously been infected have been shown to be more resistant to reinfection rather than less (Addie and others 1995).

Of the three viruses, FeLV had the greatest impact on life expectancy. Six of the seven cats that were infected when the study began died within two years, in agreement with an earlier observation that 85 per cent of viraemic cats died within 3·5 years of being naturally infected (McClelland and others 1980). The seventh infected cat died after five years. While these viraemic cats were in the household, none of the in-contact cats became viraemic, indicating that they were immune and resistant to reinfection. All but one had high titres of virus neutralising antibody indicating that they had been transiently infected, which is an excellent indicator of an immune cat (Hardy and others 1976). In this type of household, FeLV infection is therefore self-limiting, although any cats introduced would have been at great risk while the infected cats were still alive.

FIV infected an additional six cats during six years. At the last sampling in October 1998, four of the five remaining cats were infected with FIV, indicating that cats exposed to FIV do not appear to become immune and resistant to infection, as do many cats exposed to FeLV.

The present findings differ from those of Shelton and others (1990) who found no evidence of transmission of FIV in a household of 73 cats over a period of three years. A knowledge of the infectivity of FIV is important because veterinary surgeons need to know whether to advise their clients to segregate FIV-positive from FIV-negative cats. The consensus of opinion has been that FIV-infected cats are more dangerous to free-roaming cats through biting than they are to cats that live peacefully with them in the same household (Hardy 1991). The findings of other research on this subject are summarised in Table 1. The proportion of cats in contact with FIV-infected cats which became infected with FIV varied from 0 to 100 per cent. The variable transmission of the virus has been attributed to differences in the infectivity of different strains of FIV, to increased viral shedding by symptomatic cats, and to differing susceptibility to infection or different patterns of fighting among cats in the same household (Hosie and others 1989b). In the household described here there was no aggression between cats other than some paw-flailing at mealtimes. It is therefore possible that the virus may have been transmitted via saliva by mutual grooming, close contact or the sharing of food bowls. This finding contrasts with the results of a previous study which suggested that these activities were not sufficient for the transmission of FIV (Shelton and others 1989), but it is consistent with the original report of FIV in which two cats in contact with FIV-positive cats seroconverted three months after their first test (Pedersen and others 1987). On the basis of the present results it would appear to be advisable to separate FIV-infected from uninfected cats to prevent further transmission of the virus. However, since FIV-infected cats may live at least as long as their uninfected counterparts, a test and euthanasia policy to eradicate FIV from households cannot be justified, although ultimately, such decisions can only be made by the owner of the cats concerned.

The results of this study agree with other epidemiological studies of FIV which suggest that it has a long incubation period after initial primary infection and that cats may remain asymptomatic for seven years or more (Pedersen and Barlough 1991, Kohmoto and others 1998). However, in this study the FIV-infected cats appeared to survive longer after diagnosis than the cats which were FIV-negative. Although the number of cats involved was small and this difference was not statistically significant, this finding was surprising because it was expected that FIV would reduce a cat's lifespan. Although FeLV has been proposed as a potentiating co-factor in FIV infection (Pedersen and others 1990), the three cats in this household that were infected with both viruses (H, R and W) did not die any earlier than the others and cat W survived almost five years after being diagnosed. It will be important to find out whether the virus load in the four remaining cats is low, which might account for the low pathogenicity, or whether the virus is of a phenotype with a low virulence.

It was not possible in all cases to ascribe the cause of death to any of the three viruses present, so that the figures for the

longevity of the FIV-infected cats were probably reduced by cats dying of conditions other than those due to FIV. Both FeLV and FIV are directly oncogenic (Beatty and others 1998), causing T and B cell lymphomas, respectively, and they may be indirectly oncogenic by immunosuppression. Neoplastic conditions were encountered in eight of the 20 cats which died. Cat A, infected with FIV but not FeLV, had a T cell alimentary lymphosarcoma, and another FIV-infected cat (cat C) had a thymoma. Two FeLV-infected cats developed leukaemia and a third developed an osteosarcoma. Neoplasia are expected in retrovirus-infected cats but they also occurred in three of the seven FeLV- and FIV-negative cats. Transient FeLV infection has been implicated in FeLV-negative lymphosarcomas (which cat G was suffering from), but not in squamous cell carcinoma (cat F) or alimentary carcinoma (cat E).

Anorexia, pyrexia, abdominal pain and severe diarrhoea leading to death were reported in five of 10 cats experimentally coinfected with FeLV and FIV (Pedersen and others 1990). One of the cats in the present household (cat H) was euthanased because of melaena which could possibly have been attributable to the coinfection, but the diagnosis was not confirmed postmortem.

The results of this study demonstrate the dynamics of FCoV, FeLV and FIV infection in a multicat household, and the usefulness of testing cats for these viruses.

Antibody testing was useful to establish that the cats were infected with FCoV. The virus was very contagious and infected all 26 during the study; a random sampling of a few cats would therefore have revealed the infection. Repeat sampling showed that some cats eliminated FCoV infection and became seronegative, but were reinfected, possibly by a carrier cat. A significant increase in FCoV antibody titre was not a poor prognostic sign – only one of 10 cats with significantly raised antibody titres developed FIP – thus it is important not to place too much reliance on a rise in FCoV antibody titres which may not necessarily mean that the cat is going to become clinically ill. Coinfection with retroviruses did not increase a cat's chance of developing FIP.

FeLV affected fewer of the cats but was more rapidly fatal. The measurement of virus neutralising antibody titres is useful to reveal whether or not it is safe to mix FeLV-positive and negative cats. Cats with discordant FeLV results should be tested repeatedly to establish their fate. In the absence of new cats being introduced, FeLV infection died out of the household within five years.

FIV may be transmitted in households where there appears to be little or no aggression between the cats, and every attempt should therefore be made to separate FIV-infected and uninfected cats. However, FIV did not appear to affect the cats, life expectancy adversely.

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Clinical Findings and Survival in Cats Naturally Infected with Feline Immunodeficiency Virus

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Background: The clinical course and outcome of natural feline immunodeficiency virus (FIV) infection are variable and incompletely understood. Assigning clinical relevance to FIV infection in individual cats represents a considerable clinical challenge.

Objective: To compare signalment, hematologic and biochemical data, major clinical problem, and survival among client-owned, FIV-infected, and uninfected domestic cats.

Animals: Client-owned, domestic cats tested for FIV (n = 520).

Methods: Retrospective, case control study. Logistic regression analyses were conducted to identify risk factors for FIV infection and to compare hematologic and biochemical data between cases and controls, after adjusting for potential confounders. Survival times were compared using Kaplan–Meier curves.

Results: The prevalence of FIV infection was 14.6%. Mixed breed, male sex, and older age were risk factors for FIV infection. Hematologic abnormalities, biochemical abnormalities or both were common in both FIV-infected and uninfected cats. Lymphoid malignancies were slightly more common in FIV-infected than uninfected cats. Survival of FIV-infected cats was not significantly different from that of uninfected cats.

Conclusions and Clinical Importance: Multiple hematologic and biochemical abnormalities are common in old, sick cats regardless of their FIV status. Their presence should not be assumed to indicate clinical progression of FIV infection. A negative effect of FIV on survival was not apparent in this study.

Key words: Clinicopathological findings; Feline immunodeficiency virus; Survival.

Feline immunodeficiency virus (FIV) is a common pathogen of domestic cats worldwide.[1] The number of FIV-infected pet cats in the United States alone is estimated to exceed 2.5 million.[2,3] Most natural infections likely result from intercat aggression, whereas transmission from queens to kittens and between cats within stable, closed households seems to be rare.[4,5] Risk factors for infection, including male sex, intact status, outdoor access, increasing age, and concurrent health problems are well documented.[2,4,6]

Feline immunodeficiency virus is closely related to human immunodeficiency virus (HIV) with regard to its morphology, in vitro characteristics, and elements of its pathogenesis.[1] In cats experimentally infected with FIV, progressive aberrations in multiple parameters of immune function, such as lymphocyte subset counts and mitogen responsiveness, have been documented.[7] Interestingly, these changes are rarely associated with clinical signs. This may be attributed to limited exposure to secondary and opportunistic pathogens in a minimal disease setting, genetic characteristics of the host or the dose, and strain of the infecting inoculum.

Abbreviations:

AIDS	acquired immunodeficiency syndrome
ALT	alanine aminotransferase
FeLV	feline leukemia virus
FIV	feline immunodeficiency virus
HIV	human immunodeficiency virus
IFA	indirect immunofluorescence assay
IQR	interquartile range
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
OR	odds ratio
PCR	polymerase chain reaction
95% CI	95% confidence interval

Disease in HIV-infected humans without access to antiretroviral treatments is quite predictable, progressing through well-defined clinical stages: acute phase, asymptomatic carrier, persistent generalized lymphadenopathy, acquired immunodeficiency syndrome (AIDS)-related complex, and AIDS. The median time to the onset of the terminal AIDS stage is 8–10 years.[8] This stage is characterized by "AIDS-defining" illnesses, many of which are rare except in the face of profound immunosuppression (eg, *Pneumocystis* pneumonia). Disease staging includes consideration of the patient's CD4 + lymphocyte count which, together with plasma viral load, provides a surrogate marker to predict clinical outcome.[9]

The clinical course of FIV infection, on the other hand, is less well characterized or predictable. Attempts at clinical staging of FIV-infected cats have been attempted but not widely adopted.[10] A wide range of clinical signs has been reported in cats naturally infected with FIV, including oral disease, persistent cytopenias, immune-mediated disease, unexplained

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wasting, atypical, refractory or recurrent infections, and neurologic signs.[4,11] However, few of these signs have been demonstrated to be significantly different from those of control populations. With the exception of a subset of lymphomas, AIDS-defining illnesses are not recognized for FIV.[12] Furthermore, some FIV-infected cats remain asymptomatic with a normal life expectancy.[5]

The challenge for the clinician faced with a sick, FIV-infected cat is determining whether the virus is contributing to the current clinical signs. Studies comparing clinicopathological findings and outcomes between cats infected with FIV and appropriate control groups can inform our understanding of the consequences of natural infection, but such studies are limited.[13–18] The aims of this study were to compare the hematologic and biochemical changes, major clinical problem, and survival between groups of client-owned, FIV-infected and uninfected cats. Prevalence and risk factors for FIV infection also were determined.

Materials and Methods

Source of Data

The medical records of the Valentine Charlton Cat Centre, University of Sydney, were searched, using the terms FIV and feline immunodeficiency virus, for FIV testing results recorded between January 2005 and October 2009. The clinical indication for retrovirus testing had been determined by the attending clinician.

FIV and Feline Leukemia Virus (FeLV) Testing

Serology for FIV and feline leukemia virus (FeLV) was performed using commercial kits.[a,b] Polymerase chain reaction (PCR) testing for FIV was carried out at a commercial laboratory.[c] The sensitivity and specificity of this assay have been estimated to be 85–95% and 94–96%, respectively.[19] The FeLV indirect immunofluorescence assay (IFA) was performed at a commercial laboratory.[d]

Case and Control Definitions

A cat was defined as "FIV-infected" if it tested seropositive for FIV and had not been vaccinated,[e] as determined from the medical record or direct owner communication. A cat was considered to be "FIV-uninfected" if it tested seronegative or it tested seropositive and had been vaccinated but had returned a negative result on FIV PCR testing. FIV seropositive, vaccinated cats with unknown PCR status and seropositive cats with unknown vaccination and PCR status were excluded. A FeLV antigen test was considered to be positive if a positive result on in-house testing was confirmed by IFA, or the cat was in contact with an antigenemic cat.

Data Collection

Information obtained from the medical record including breed, sex, neuter status, date of FIV testing, FeLV antigen status (where tested), and date of death were recorded for FIV-infected (n = 76) and FIV-uninfected (n = 444) populations. The first hematologic and biochemical data, performed by Veterinary Pathology Diagnostic Services, University of Sydney, subsequent to FIV testing were recorded for FIV-infected cats (n = 75, data unavailable for 1 cat) and a subset of the control population (n = 231) that was selected using random numbers.[f] The median time lag between testing and hematologic and biochemical data collection was 0 days for both FIV-infected and control groups (FIV infected; range, 0–4179 days; interquartile range [IQR], 179; uninfected; range, 0–232 days; IQR, 1). The major clinical problem in these cats was assigned to 1 of 10 categories: cardiorespiratory, endocrine, gastrointestinal, genitourinary, healthy, immune-mediated, infectious, neoplasia, neurologic or not determined. Within the neoplasia category, the prevalence of lymphoid versus other malignancies was determined.

Data Analysis

Statistical software was used for all analyses.[g] All *P* values were 2-sided and considered significant at <.05. For risk factor and survival analyses, data from 520 cats were used, 76 infected cats and 444 uninfected controls. For analysis of analytes, data from 306 cats were used, 75 infected cats and 231 uninfected controls. Descriptive analyses were conducted to understand the distribution of variables and their preliminary association with FIV status.

Three sets of logistic regression analyses then were performed. The 1st set of analyses was conducted to identify any association between "FIV status" and the demographic factors "breed", "sex", "neuter status", and "age at FIV testing". Similar logistic regression analyses were conducted to evaluate the association between "FIV status" and hematologic and biochemical variables. To further compare the hematologic and biochemical data between FIV-infected and control cats, each hematologic and biochemical value was classified as decreased, normal or increased for each cat and a 3rd set of logistic regression analyses was conducted to compare analyte concentrations between FIV-infected and uninfected cats. Age and sex of cats were considered potential confounders and forced into the models for hematologic and biochemical variables, even if not significant. Univariable and multivariable model building was performed [http://sydney.edu.au/vetscience/biostat/macros/multi_about.shtml].[20]

The major clinical problem was compared between FIV-infected cats and the control sample using the 2-tailed Fisher's exact test. The only FeLV antigenemic cat had lymphoma and was excluded from analysis of major clinical problem.

Two survival analyses using the Kaplan–Meier approach were conducted to compare survival between FIV-infected and uninfected cats. The 1st analysis compared the age at the time of data collection (ie, date of death or censoring − date of birth) whereas the 2nd analysis compared survival time at the time of data collection (ie, date of death or censoring − date of testing). All surviving cats were censored at the date of their last visit to the clinic or at the time of data collection (August 17, 2010), whichever was earlier. Log rank test was used for comparisons.

Results

FIV and FeLV Testing

Five hundred twenty-five cats were tested for FIV during the study period. Seventy-six FIV seropositive cats that had not been vaccinated against FIV were considered to be FIV-infected. Five cats that tested seropositive for FIV but with undetermined vaccination status tested negative on PCR and were considered to be FIV-uninfected. The infection status

of 5 FIV seropositive cats could not be determined and they were excluded. The 439 cats that were seronegative were considered to be FIV-uninfected. In total, 76 FIV-infected cats and 444 FIV-uninfected cats were available for study. The prevalence of FIV was 14.6%. A single, FIV-uninfected cat was positive for FeLV antigen giving a prevalence of less than 0.2%.

Analysis of Risk Factors of FIV Infection

The mean age at testing was 9.8 (±4.3) years and 7.8 (±5.2) years for FIV-infected and uninfected groups, respectively. Mixed breed, male and neutered cats made up 88.2, 76.3, and 5.3% of the infected group in comparison to 66.2, 51.1, and 6.8%, respectively, of the uninfected group. The final multivariable model had three significant variables, "age at FIV testing", "sex", and "breed". The assumption of linearity for "age at FIV testing" was invalid, therefore, it was split into 4 categories: age ≤5 years, >5–10 years, >10–15 years, and >15 years. Results for the final model demonstrated that the risk of being FIV-infected was greater for cats over 5 years of age than for cats of 5 years of age or younger. Female cats (odds ratio [OR], 0.30; 95% CI, 0.17, 0.53) and purebred cats (OR, 0.28; 95% CI, 0.13, 0.56) were less likely to be FIV positive.

Analysis of Hematologic and Biochemical Data

Hematologic and biochemical results were analyzed for potential associations with FIV status. Of the 33 analytes evaluated, 9 had *P* values <.25 in univariable logistic regression analyses (Table 1). After adjusting for potential confounders, age and sex, only sodium was significant in the final logistic regression model. The assumption of linearity for sodium was not valid, therefore the cubic spline was fitted (data not shown). The results indicated that the log odds of being FIV-infected is increased as the sodium concentration increased above 150 mmol/L.

Comparison of Hematologic and Biochemical Parameters for FIV-Infected and FIV-Uninfected Cats with Normal Range for Each Analyte

Logistic regression analyses were conducted by categorizing all hematologic and biochemical parameters into three categories: decreased, normal, and increased. Of the 33 analytes evaluated, 11 were significant at a liberal *P*-value of .25 (Table 2). PCV, chloride, MCH, and MCHC were excluded from further analyses because of 0 or low frequencies for some cells. Only plasma sodium concentration and monocyte count were significant in the final model after adjusting for age and sex (Table 3). Compared with controls, the cases had greater odds of hypernatremia and decreased odds of hyponatremia. FIV-infected cats were at increased risk of monocytopenia (Table 3).

Hematologic and clinicopathological abnormalities that may be attributed to FIV infection, when it is present, are presented in Table 4. There was no significant difference in the frequency of these abnormalities between infected and control groups. Uninfected cats were as likely, or more likely, to be leukopenic, lymphopenic, hyperproteinemic, hyperglobulinemic, and azotemic than FIV-infected cats.

Table 1. Summary statistics of the association of hematological and biochemical parameters with FIV status.

Variable	Status	N	Minimum	Lower Quartile	Median	Upper Quartile	Maximum	*P*-value
PCV (L/L)	FIV-infected	50	0.16	0.28	0.33	0.37	0.44	.052
	FIV-uninfected	162	0.05	0.25	0.31	0.36	0.46	
Hb (g/L)	FIV-infected	51	50.0	95.0	116.0	127.0	161.0	.057
	FIV-uninfected	167	6.6	89.0	107.0	124.0	160.0	
MCV (fl)	FIV-infected	50	37.1	43.3	46.05	49.7	60.2	.074
	FIV-uninfected	153	33.1	41.4	44.4	46.7	84.8	
MCH (pg)	FIV-infected	50	13.5	14.7	15.8	17.2	19.5	.006
	FIV-uninfected	160	1.6	14.05	15.2	16.3	24.2	
MCHC (g/L)	FIV-infected	50	312.0	335.0	343.0	353.0	400.0	.21
	FIV-uninfected	166	24.0	331.0	341.5	355.0	438.0	
Albumin (g/L)	FIV-infected	38	13.3	26.8	29.7	33.4	39.3	.022
	FIV-uninfected	131	7.43	29.3	32.5	34.7	43.0	
Cholesterol (mmol/L)	FIV-infected	36	1.8	2.8	3.4	4.5	137.0	.097
	FIV-uninfected	129	1.6	2.9	3.5	4.7	137.0	
CK (U/L)	FIV-infected	34	11.0	126.0	221.5	330.0	1323.0	.18
	FIV-uninfected	125	52.0	111.0	197.0	345.0	12726.0	
Sodium (mmol/L)	FIV-infected	35	132.4	146.4	151.5	155.8	162.1	<.001
	FIV-uninfected	130	126.4	139.7	144.6	148.7	172.6	

The *P*-values are for likelihood ratio chi-square test based on univariable logistic regression anlayses. Results are presented for only variables with *P*-value <.25.

Variables also examined but not significant ($P > .25$) were absolute erythrocyte reticulocyte, leukocyte, neutrophil (segmented and band), monocyte, eosinophil, basophil, lymphocyte and platelet counts, inorganic phosphate, glucose, creatinine, urea, total calcium, alanine aminotransferase, alkaline phosphatase, bilirubin, total protein, globulin, potassium, and chloride.

Table 2. Contingency tables of categorized hematologic and biochemical variables with FIV status.

Variables	Categories	FIV-infected (%)	FIV-uninfected (%)	Total	*P*-value
Sodium (mmol/L)	Decreased (≤147)	10 (28.6%)	81 (62.1%)	91	<.001
	Normal (>147–156)	18 (51.4%)	44 (33.9%)	62	
	Increased (>156)	7 (20%)	5(3.9%)	12	
Chloride (mmol/L)	Decreased (≤115)	7 (20%)	64 (50.8%)	71	.001
	Normal (>115–130)	28 (80%)	62 (48.4%)	90	
	Increased (>130)[a]	0 (0.0%)	2 (1.6)	0	
MCH (pg)	Decreased (≤13)[b]	0 (0.0%)	16 (10.0%)	16	.003
	Normal (>13–17)	36 (72.0%)	125 (78.1%)	161	
	Increased (>17)	14 (28.0%)	19 (11.9%)	33	
Monocytes × 10^9/L	Decreased (≤0.08)	8 (15.4%)	8 (5%)	16	.03
	Normal (>0.08–0.56)	33 (64.5%)	98 (61.3%)	131	
	Increased (>0.56)	11 (21.1%)	54 (33.8%)	65	
Bilirubin (μmol/L)	Decreased (≤2.5)	11 (57.9%)	37 (32.5%)	48	.11
	Normal (>2.5–3.5)	4 (21.1%)	35 (30.7%)	39	
	Increased (>3.5)	4 (21.1%)	42 (36.8%)	46	
Creatinine (μmol/L)	Decreased (≤90)	4 (9.5%)	24 (16.9%)	28	.17
	Normal (>90–180)	27 (64.3%)	97 (68.3%)	124	
	Increased (>180)	11 (26.2%)	21 (14.8%)	32	
Hb (g/L)	Decreased (≤80)	3 (5.9%)	27 (16.2%)	30	.11
	Normal (>80–140)	45 (88.2%)	128 (76.7%)	173	
	Increased (>140)	3 (5.9%)	12 (7.2%)	15	
MCV (fl)	Decreased (≤40))	6 (12%)	26 (17%)	32	.14
	Normal (>40–45)	15 (30%)	63 (41.2%)	78	
	Increased (>45)	29 (58%)	64 (41.8%)	93	
Calcium (mmol/L)	Decreased (≤1.75)	3 (7.3%)	2 (1.4%)	5	.18
	Normal (>1.75–2.6)	27 (65.9%)	101 (72.7%)	128	
	Increased (>2.6)	11 (26.8%)	36 (25.9%)	47	
PCV (L/L)	Decreased (≤0.30)	19 (38.0%)	80 (49.4%)	99	.15
	Normal (>0.30–0.45)	31 (62.0%)	81 (50.0%)	112	
	Increased (>0.45)[a]	0 (0.0%)	1 (0.6)	1	
MCHC (g/L)	Decreased (≤310)[b]	0 (0.0%)	8 (4.8%)	8	.18[b]
	Normal (>310–350)	37 (74%)	104 (62.7%)	141	
	Increased (>350)	13 (26%)	54 (32.5%)	67	

The *P*-values are for likelihood ratio chi-square test based on univariable logistic regression anlayses. Results are presented for only variables with *P*-value <.25.

[a]These categories were excluded from logistic regression analyses because of very small frequencies.

[b]The *P*-values are for Fisher's exact test as logistic regression model could not converge because of some zero cell frequencies.

Table 3. The final logistic regression model to evaluate association of categorized hematological and biochemical parameters with FIV status.

Variables	Categories	b	SE	Adjusted Odds Ratios	95% Confidence Intervals	*P*-value
Intercept		−3.50	0.91			
Sodium	Normal (>147–156)	0.00		1.00		.001
	Decreased (≤147)	−1.04	0.48	0.35	0.13, 0.89	
	Increased (>156)	1.89	0.83	6.63	1.41, 38.11	
Monocytes	Normal (>0.08–0.56)	0.00		1.00		.035
	Decreased (≤0.08)	1.96	0.81	7.13	1.50, 37.18	
	Increased (>0.56)	−0.14	0.51	0.87	0.31, 2.34	
Gender	Female	0.00		1.00		.017
	Male	1.24	0.52	3.44	1.31, 10.10	
Age at diagnosis	≤5 years	0.00		1.00		.053
	>5–10 years	1.60	0.86	4.95	1.08, 36.56	
	>10–15 years	2.25	0.85	9.51	2.16, 70.34	
	>15 years	1.14	0.99	3.14	0.49, 27.14	

Odds ratios are adjusted for other variables in the model. For example, compared to FIV uninfected cats, FIV infected cats had 6.63 times odds of having increased sodium concentrations and 7.13 times odds of decreased monocyte counts.

Table 4. Comparison of abnormalities commonly attributed to FIV infection in infected and uninfected cats.

	FIV-infected	FIV-uninfected
Abnormality	Affected/total (%)	Affected/total (%)
Leukopenia	21/52 (40.4)	66/161 (40.9)
Neutropenia	20/52 (38.5)	54/161 (33.5)
Lymphopenia	27/53 (50.9)	80/162 (49.4)
Hyperproteinemia	20/50 (40)	74/152 (48.7)
Hyperglobulinemia	8/37 (21.6)	30/129 (23.3)
Increased creatinine	4/42 (9.5)	24/142 (16.9)
Increased urea	7/43 (16.3)	33/145 (22.8)

There was no significant difference in these variables between FIV-infected and uninfected populations.

Table 5. The major clinical problem in FIV infected and uninfected cats.

Major clinical problem	FIV-infected (n = 75)		FIV/FeLV-uninfected (n = 230)		*P*
Cardiorespiratory	5	6.7%	18	7.8%	1.0
Endocrine	4	5.3%	15	6.5%	1.0
Gastrointestinal	10	13.3%	36	15.6%	.7
Genitourinary	4	5.3%	9	3.9%	.5
Healthy	4	5.3%	15	6.5%	1.0
Immune mediated	3	4.0%	12	5.2%	1.0
Infectious	10	13.3%	24	10.4%	.5
Neoplasia (total)	24	32.0%	55	23.8%	.2
lymphoid neoplasia	16	21.3%	30	13%	.1
Neurological	4	5.3%	20	8.6%	.5
No final diagnosis	7	9.3%	26	11.7%	.8

Comparison of Major Clinical Problem between FIV-Infected and Uninfected Cats

The major clinical problems identified in FIV-infected cats and the control sample are presented in Table 5. Almost 95% of all cats tested for FIV presented with clinical problems. In both groups, the most common clinical problems were neoplastic and gastrointestinal diseases and no significant differences between the groups were identified. Among cases of neoplasia, lymphoid malignancies were slightly more common in FIV-infected cats (16/75, 21.3%) than uninfected cats (30/230, 13%).

Comparison of Survival Time between FIV-Infected and Uninfected Deceased Cats

Thirty-eight FIV-infected and 134 uninfected cats died during the study period. Kaplan–Meier survival curves are shown in Figure 1. There was no difference in survival age ($P = .8$, log-rank test) or survival time ($P = .4$, log-rank test) between FIV-infected cats and uninfected cats.

Discussion

In this study, we combined analysis of hematologic and biochemical changes, major clinical problem and outcome in client-owned cats tested for FIV. FIV-infected cats were compared with an uninfected control group adjusted for age and sex. The prevalence of FIV in this group of predominantly sick cats was 14.6%, which is in accordance with previous studies of sick cats from the Asia Pacific region where FIV prevalence data are consistently among the highest found internationally.[21,22] In contrast, the finding of a single cat with FeLV antigenemia among 288 cats tested is consistent with the very low prevalence of FeLV in Australia.[23] Analysis of risk factors for FIV infection identified that mixed breed, male cats were more likely to be infected than purebred, female cats. Age also was a risk factor with older cats (>5 years old) being 4 times more likely to be FIV-infected than younger cats (≤5 years old). Similar risk factors have been reported worldwide demonstrating that our group displays characteristics typical for FIV-infected cat populations.[2,4,6,17]

A substantial proportion of FIV-infected cats was anemic (38%), lymphopenic (50.9%), or hyperproteinemic (40%). However, similar trends were observed in FIV-uninfected cats where 49.4% were anemic, 49.4% lymphopenic, and 48.7% hyperproteinemic. Multiple hematologic and biochemical abnormalities have been

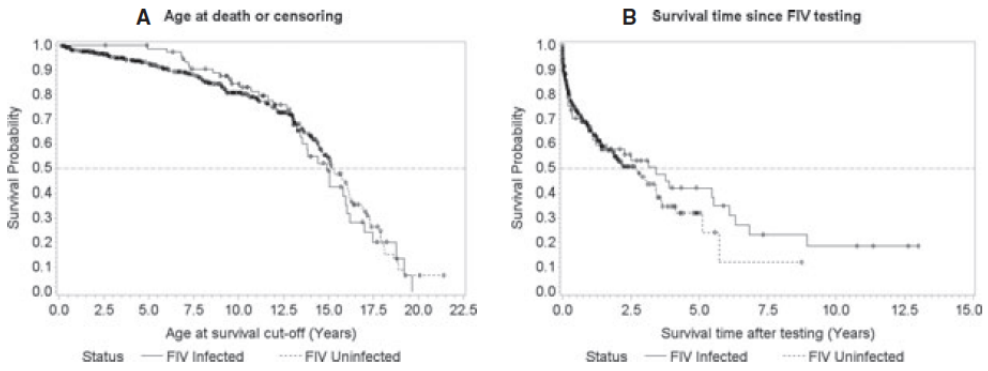

Fig 1. Kaplan–Meier curves showing survival of FIV-infected and FIV-uninfected cats. Curves for FIV-infected and uninfected groups indicate the proportion of surviving cats in each group at a given age (A) or after a given time after testing (B).

described as occurring commonly in FIV-infected cats, although matched, uninfected cats were not included in these early studies.[4,11,24–26] This highlights the importance of including a control sample when attempting to ascribe clinical relevance to such observations.

Significant differences in serum sodium concentrations were observed between infected and noninfected cats. The majority of controls were hyponatremic. Hyponatremia is the most common electrolyte disorder in sick humans and results from a diverse range of disease states and interventions.[27] These include liver disease, renal disease, vomiting, diarrhea, congestive heart failure, diuretic treatment, and hypotonic fluid administration.[28] FIV-infected cats were as likely to be hyponatremic as hypernatremic but, interestingly, infected cats were much less likely than controls to be hyponatremic. As the investigation of factors affecting sodium balance in individual cats was beyond the scope of this study, we can only speculate as to why there may be a decreased risk of hyponatremia in FIV infection. One explanation is a tendency for hypernatremia in infected cats that offsets hyponatremia seen in uninfected, sick cats. Significantly increased plasma sodium concentrations were reported in FIV-infected cats from 43 months postexperimental infection.[29] Among field cases, hypernatremia was present in 6% of 48 FIV-infected cats.[24] Hypotonic fluid losses through vomiting, diarrhea, fever, renal compromise, and decreased water intake can contribute to increased plasma sodium concentrations. Renal diseases are suspected in FIV infection, but a causal association has been difficult to prove.[30] We found no difference between FIV-infected cats and controls in plasma creatinine concentration, and genitourinary diseases were not a major problem in either group. Thirst could be decreased in FIV infection by a central effect, because some FIV isolates are neurotropic, or secondary to cognitive dysfunction, similar to AIDS dementia.[31,32]

An increased risk of hyperglobulinemia was reported in 2 controlled studies of natural FIV infection.[13,15] This likely reflects polyclonal B cell expansion, which is a hallmark of HIV infection in humans and has been documented in both natural and experimental FIV infection.[33,34] In experimentally infected cats followed longitudinally, plasma globulin concentration increased up to, but not after, 4.5 years postinfection.[29] It was postulated that this observation was because of the eventual onset of B cell loss. Advanced FIV infection is characterized by profound lymphoid depletion.[10,35,36] In a cross-sectional study of natural infection, Walker et al found lower proportions of B lymphocytes in cats with advanced disease compared with those at earlier stages.[37] In our study, hyperglobulinemia was seen in 21.6% of FIV-infected cats and in a similar proportion (23.3%) of uninfected cats. The mean age at diagnosis of FIV-infected cats was 9.8 years and it is possible that many had been infected for years, which might explain why no association with increased plasma globulin concentration was identified. Thomas and others reported a similar finding.[15] They demonstrated significant lymphopenia and hypergammaglobulinemia in cats naturally infected with FIV compared with controls, but when this relationship was analyzed in relation to age, it was found that neither variable was associated with FIV in cats >8 years of age.

FIV infection carried an increased risk of monocytopenia. Walker and Canfield also reported significant monocytopenia in FIV-infected pet cats compared with clinically matched, uninfected cats.[37] Bone marrow examinations of cats in this study identified a normal or proliferating myeloid pool. In cats with terminal illness, FIV sequences were found predominantly in cells of the monocyte/macrophage lineage raising the possibility of a direct viral effect on monocyte maturation as a cause of monocytopenia.[38]

Direct comparison between controlled field studies is hampered by differences in study populations, data collection, and analyses. Notwithstanding these differences in study populations and design, when data from controlled field studies, including ours, are considered as a whole no hematologic deficits have been consistently associated with FIV infection.[13–16,18,39] Thus, although retrovirus testing is indicated in the investigation of hematologic abnormalities, their presence in a sick, FIV-infected cat should not be interpreted as evidence that the prognosis for that cat is worse, compared with an uninfected cat with similar hematologic findings. For example, a number of abnormalities have been described in FIV-infected cats that could contribute to anemia, including decreased or aberrant erythroid maturation and hemostatic abnormalities.[18,25,40] However, anemia is a complex, multifactorial problem and its cause or causes may not always be identified in a sick cat with multiple problems. The fact that no other cause has been identified in an anemic patient infected with FIV does not imply that the problem is necessarily a consequence of FIV infection.

In 2 of 5 FIV-infected cats, the major clinical problem was lymphoid malignancy. Several lines of evidence support that, just as in HIV infection, there is a group of lymphoproliferative malignancies associated with FIV infection. An increased risk of developing lymphoma in natural FIV infection has been demonstrated.[41] Histopathological and immunohistochemical studies describe high-grade, B cell, extranodal neoplasms, features characteristic of HIV-associated lymphomas.[42] It will be important to further characterize malignancies arising in FIV-infected cats in the field to understand the spectrum of relationships between FIV and neoplasia and their etiologies.

The survival time was comparable between FIV-infected and uninfected cats. This contradicts a still widely held belief that FIV infection confers decreased life expectancy, but is in agreement with recent case control studies investigating similar numbers of FIV-infected pet cats as described in our study.[6,17] Similarly, survival in cats experimentally infected with FIV over a 6.5 year period (10/10) was comparable with that in uninfected controls (9/10).[29] In the largest study of almost 10,000 retrovirus tested pet cats, including 1100 seropositive for FIV, the survival rate at 6 years was

65% compared to 90% for uninfected cats.[43] Interestingly, if deaths during the first 100 days were excluded, survival of FIV-infected cats was 94 and 80% at 3 and 6 years, respectively, compared with controls. There is evidence that euthanasia based on the diagnosis of FIV infection may contribute to an observation of decreased survival in studies of FIV-infected cats. First, an investigation of risk factors for mortality in United Kingdom cat adoption centers found that, although FIV was the major single reason for euthanasia, no natural deaths could be attributed to this infection.[44] Second, Ravi and others reported that, of 58 FIV seropositive cats studied, 17 were euthanized at testing and in 9 of those the reason was the positive test result itself, rather than a specific clinical problem.[17]

The in-house testing kits used here perform well with sensitivities and specificities for FIV antibody detection approaching 100% when compared with western blot or with each other.[21,45] Confirmatory western blot testing was not performed but, as the results would be expected to vary little from serology, its value is questionable. The definitions of FIV-infected and FIV-uninfected used here combine history with results of serologic and, where indicated, molecular testing. This approach is necessary because of seroconversion following vaccination. Although it introduces potential errors in determining infection status, any such errors could have affected only a small proportion of cases reported here. The prevalence of FIV may have been higher than the 14.6% reported. Five cats that tested seropositive for FIV but with uncertain vaccination status, tested negative on PCR and were considered to be FIV-uninfected. This assumption may be false. It is not possible to eliminate the potential for vaccine-induced rather than infection-associated antibody in all cases. The sensitivity of PCR methodologies for detecting FIV is expected to be less than that of serology. The reported estimate of sensitivity of the PCR tests used here is similar, although lower, than estimates for serology.[19] Virus isolation after cocultivation of peripheral blood mononuclear cells is not practical to use as a confirmatory test because it is not commercially available and is not applicable to retrospective data sets. On the other hand, exclusion of another 5 seropositive cats of uncertain infection status may have falsely decreased the prevalence. A requirement for supportive evidence for defining FeLV antigen positive cats was imposed here because of the low prevalence of FeLV in this area and the subsequent poor positive predictive value of in-house tests.[23]

There are limitations to our study. The control population comprising cats 'at-risk' for FIV infection was selected because of its clinical relevance. These controls were crucial in identifiying the similarity of clinical abnormalities detected in cats tested for FIV, regardless of the outcome of the test. This control group is unlikely to be representative of the total population of FIV-uninfected cats. The quality of data from retrospective studies is limited by nonstandardized collection and incomplete data sets. The recording of the major clinical problem carries an element of subjectivity and does not account for the presence of multiple problems. The clinical consequences of FIV infection may be subtle and inconsistently detected at a population level, an issue that has hindered demonstration of pathogenicity of FIV strains infecting nondomestic species.[46] Many FIV-infected cats were censored from the survival analysis because they were still alive at the time of completion of the study and this should be noted when interpreting the data. Despite these drawbacks, studies of natural infection provide information relevant for practitioners faced with sick, FIV-infected cats.

Initial reports implying that FIV infection by itself imparts a poor prognosis should be interpreted with caution. Until surrogate markers for FIV disease progression are validated in longitudinal studies of naturally infected cats, the prognosis for an individual FIV-infected cat should be determined without regard to its FIV status.

Footnotes

[a] FIV, FeLV Rapid Immunomigration, AGEN Biomedical Ltd, Acacia Ridge, QLD, Australia
[b] Snap Combo, IDEXX Laboratories, Zetland, NSW, Australia
[c] Gribbles Veterinary Pathology, Clayton, VIC, Australia
[d] Vetpath Laboratory Services, Ascot, WA, Australia
[e] Fel-O-Vax FIV, Boehringer Ingelheim, Germany
[f] Microsoft Excel RAND function, 2007, Microsoft Corp, Redmond, WA
[g] SAS statistical software, release 9.3, 2002–10, SAS Institute Inc, Cary, NC

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Contrasting clinical outcomes in two cohorts of cats naturally infected with feline immunodeficiency virus (FIV)

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ABSTRACT

Despite over 25 years of feline immunodeficiency virus (FIV) research, relatively little is known about the longitudinal course of FIV infection following natural infection. In contrast to published reports of experimental infections using lethal strains of the virus, clinical signs of naturally acquired FIV infection can be mild or inapparent, rather than life-threatening. In this prospective, longitudinal controlled study, based in Chicago, IL ($n = 17$) and Memphis, TN ($n = 27$), we investigated two cohorts of privately owned, naturally infected cats kept under different housing conditions. Cats in the Chicago cohort (Group 1) were kept in households of $\leq$2 cats, while the Memphis cohort (Group 2) comprised part of a large multi-cat household of over 60 cats kept indoors only, with unrestricted access to one another.

The majority of cats from Group 1 did not display clinical signs consistent with immunodeficiency during the 22-month observation period. In contrast, the outcome of infection in Group 2 was dramatically different; 17/27 (63%) of cats lost a median of 51.3% of their bodyweight ($P < 0.0005$) and died during the study period, with lymphoma being the most common cause of mortality.

Although the decrease in CD4+ T cell count between enrolment and terminal disease was significant ($P = 0.0017$), the CD4:CD8 ratio at the time of enrolment did not reliably distinguish FIV-positive cats classified as 'healthy' and 'not healthy' at either cohort. FIV load at enrolment was significantly lower in Group 1 than in Group 2 ($P < 0.0001$), but there were no significant differences at enrolment between healthy and not healthy cats at either group.

In conclusion, the results of this study suggest that management and housing conditions impact on disease progression and survival times of FIV-positive cats.
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1. Introduction

Feline immunodeficiency virus (FIV) is an important pathogen of domestic cats that is distributed worldwide. Prevalence estimates vary from 1% to 44%, depending on the geographical location as well as the entry criteria of

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each study (Hartmann, 1998; Ishida et al., 1989). Outdoor, adult, male, sick cats with bite wounds are at increased risk of infection, since transmission is mainly via bites from infected cats (Hartmann, 1998). Clinical signs are similar to those described for human immunodeficiency virus-acquired immune deficiency syndrome (HIV-AIDS) and include signs of immunosuppression, hematopoietic changes and neoplasia (Hartmann, 2011). The progression of experimental FIV infection can be monitored using the staging system used in HIV infection: acute, persistent generalized lymphadenopathy, asymptomatic carrier phase (AC), AIDS-related complex (ARC) and AIDS (English et al., 1994). While this classification is useful in well-controlled, experimental settings, it has limitations under field conditions, mainly because of strain-related variability in pathogenicity, variation in the ages of cats at the time of infection as well as possible exposure to secondary pathogens (Hartmann, 2012; Pedersen et al., 2001; Podell et al., 1997). Hence, there have been variable morbidity and mortality rates reported in naturally infected cats (Addie et al., 2000; Hofmann-Lehmann et al., 1997; Kohmoto et al., 1998; Liem et al., 2013).

Providing an accurate prognosis for FIV-positive cats is important so that optimal care can be provided by veterinarians and cat owners. Surrogate markers for HIV infection are well established and valuable in monitoring both responses to therapy and the likelihood of disease progression (Churchill, 1997; Katzenstein, 2003; Okonji et al., 2012; Shaunak and Teo, 2003; Smith and Stein, 2002). In contrast, to our knowledge, prospective controlled studies investigating the course of FIV infection and markers of disease progression have not been described. Consequently, the longitudinal course of naturally acquired FIV infection is poorly described and the variability in rates of progression to immunodeficiency are poorly understood.

In this controlled study, we examined clinical and laboratory parameters from two cohorts of cats living in different locations and kept under different housing conditions. The aim of the study was to report clinical findings, post-mortem findings, bodyweight, CD4+ T cell counts, FIV load and phylogenetic classification of viral envelope genes in two groups of cats naturally infected with FIV.

2. Materials and methods

2.1. Cats

Forty-four neutered, FIV-positive (SNAP FIV/FeLV Combo Test (IDEXX Laboratories, Westbrook, MN) cats were enrolled, with an equal number of age- and sex-matched FIV-negative cats from the same geographical locations. In order to obtain an adequate sample size FIV-positive cats and matched control cats during the 2-year enrolment period, it was necessary to source cats from two separate locations. FIV-positive results were further confirmed by virus isolation (Hosie et al., 2009). All cats were feline leukaemia virus (FeLV) antigen negative at enrolment. The number of cats enrolled into the study was capped at 44 due to the capacity of the laboratory at which molecular analyses were performed.

Seventeen of the 44 FIV-positive cats enrolled (Group 1) had been previously adopted from a large metropolitan adoption-guarantee shelter (PAWS Chicago) and lived in single-cat households in Chicago, IL, USA except for seven cats: two cats (C7 and C4) cohabited in a two-cat household; one cat (C13) lived in a two-cat household with a FIV-negative cat enrolled in the study; one cat (C9) was housed at PAWS Chicago for the first 11 weeks of the study and then was adopted into a house with an FIV-positive cat not enrolled in the study; and three cats (C2, C15 and C21) were housed at PAWS Chicago in a room containing up to three FIV-positive cats before they were each adopted into single cat households at 2, 14 and 58 weeks after enrolment, respectively.

The remaining twenty-seven FIV-positive cats enrolled (Group 2) were housed together in a large multi-cat household operating as an FIV-positive cat rescue in Memphis, TN, where a total of 53 FIV-positive and 10 FIV-negative cats were housed indoors with unrestricted access to one another. None of 10 FIV-negative cats from the Memphis cat rescue died during the study period. Since these cats were not age- or sex-matched for any of the enrolled FIV-infected cats from the same household (Group 2), their health status was not monitored using the study protocol. All enrolled FIV-negative cats at both locations lived in households of three cats or fewer.

The study and its aims were reviewed and approved by University of Glasgow Ethics Committee and the Purdue Animal Care and Use Committee. Cat owners provided written informed consent for their participation in the study.

2.2. Study timeline and collection dates

At enrolment, all cats underwent a general physical examination by a registered specialist in feline medicine (AL) and blood was collected for determination of FIV status and laboratory analysis. Oral examinations were conducted as part of the physical examination and a gingival score (0–3/3; Lobprise, 2007) was assigned. At the time of enrolment, FIV-positive cats were classified as 'healthy' if there were no abnormalities on physical examination and their gingival score was 0/3 or 1/3. Cats were classified as 'not healthy' if at least one clinical abnormality was detected, or if their gingival score was 2/3 or 3/3. The clinical classification at enrolment was based solely on physical examination findings and remained with each cat for the 22-month study period, regardless of any further changes. An entry criterion for age- and sex-matched FIV-negative cats was healthy status at the enrolment examination.

Serial physical examinations and specimen collections were made at 6-monthly intervals in FIV-positive cats over the study period. Physical examinations and specimen collections were performed at enrolment and 12 months later in FIV-negative cats. The date of the first known positive FIV ELISA test was obtained for all FIV-positive cats and the time between first diagnosis and study enrolment (months) was

calculated. Abnormalities detected during the clinical examinations are listed in Table 1.

2.3. Laboratory and post-mortem examinations

Flow cytometric analyses were performed as previously described (Lin and Litster, 2013). FIV loads were measured using a commercially available PCR test (IDEXX FIV RealPCR Test, IDEXX Laboratories, West Sacramento, CA). The assay detects the presence of viral nucleic acid, including both genomic DNA and viral RNA, in peripheral blood leukocytes with 80.5% sensitivity and 99.9% specificity (IDEXX Laboratories, West Sacramento, CA).

Post-mortem examinations were performed by a specialist veterinary pathologist (TLL).

2.4. Phylogenetic analysis

Maximum likelihood (ML) phylogenetic trees were constructed in MEGA5 (Tamura et al., 2011) under the HKY nucleotide substitution model, selected through jMODELTEST (Posada, 2008). Statistical support for the ML trees was estimated using 1000 bootstrap replicates (Efron et al., 1996). Multiple *env* sequences ($n = 355$), from cats enrolled in the study were subjected to rigorous recombination testing as described previously (Bęczkowski et al., 2014).

2.5. Statistical analysis

Graphing and statistical data analyses were performed using commercially available software (GraphPad Prism version 5.00, GraphPad Software). Descriptive data were shown as medians and interquartile range (IQR; median, 5th and 95th quartiles). Given the relatively small sample size, and after inspection of the data distributions, Mann–Whitney and Wilcoxon matched pairs tests were used to test hypotheses regarding differences in laboratory parameters between and within cat groups. Binary data were analyzed using Fisher's exact test. FIV load data were tested for correlation using Spearman correlation tests. Kaplan–Meier curves were compared using the Mantel–Cox 'log-rank' test and tested with the log-rank test for trends. Significance was set at $P < 0.05$.

3. Results

3.1. Cats

Table 2 provides breed, gender, age and time since first FIV-positive test data for all FIV-positive cats and for cats from Group 1 and Group 2. Each matching pair of FIV-positive and FIV-negative cats was aged within 2 years of each other. All enrolled cats remained FeLV-negative over the study period and none of the FIV-negative cats seroconverted.

3.2. Health status

In the FIV-positive group at the time of enrolment, there were equal numbers of healthy ($n = 22$) and not healthy animals ($n = 22$). In Group 1, 10 cats were classified as healthy (59%) and seven cats were classified as not healthy (41%); all but one cat remained in this classification during the observation period. In Group 2, 12 cats were initially classified as healthy (44%) and 15 cats that were classified as not healthy (56%) remained in these classifications throughout the study. However, 63% of cats (17/27) from Group 2 experienced severe weight loss and died during the study period. Half (6/12) of the cats classified as healthy and 73% (11/15) of cats classified as not healthy died during the study ($P = 0.26$). Almost half of cats diagnosed at post-mortem with lymphoma (44%; 4/9) had been classified as healthy at the time of enrolment (Table 3).

3.3. Post-mortem findings

During the 22-month study period, 1/17 (5.9%) FIV-positive cats from Group 1 and 17/27 (63%) FIV-positive cats from Group 2 died. Fig. 1 illustrates a Kaplan–Meier survival plot of both cohorts. The cats that died were examined post-mortem and the findings are shown in Table 3. In Group 2, post-mortem data were not available for one cat (M33).

The most common pathological finding at post-mortem in Group 2 was lymphoma; various anatomical types were documented in 9/16 (56%) cats from Group 2 (Table 4). Four cats had lymphoma limited to a single site (retrobulbar site in one case and bone marrow in three cases), while in five other cats, varying degrees of dissemination

Table 1
Clinical abnormalities detected on physical examination in FIV-positive cats ($n = 44$) over the 22-month study period.

Description	Number of cats with clinical abnormalities (%)		
	Group 1 ($n = 17$)	Group 2 ($n = 27$)	All FIV-positive cats ($n = 44$)
Dental/oral cavity (faucitis)	12 (71)	19 (70)	31 (70)
Skin (alopecia, dermatitis)	10 (59)	12 (44)	22 (50)
Ears	3 (18)	4 (15)	7 (16)
Ocular disease	2 (12)	5 (19)	7 (16)
Cardiovascular	3 (18)	2 (7)	5 (11)
Digestive tract	0 (0)	4 (15)	4 (9)
Respiratory	0 (0)	2 (7)	2 (5)
Lymph nodes	0 (0)	0 (0)	0 (0)
Urogenital	0 (0)	0 (0)	0 (0)
Neurologic	0 (0)	0 (0)	0 (0)
Musculoskeletal	0 (0)	0 (0)	0 (0)
Weight loss/anorexia	0 (0)	22 (81)	22 (50)

Table 2
Breed, gender, age and age at first known positive feline immunodeficiency virus (FIV) ELISA test at enrolment.

	Group 1 ($n = 17$)	Group 2 ($n = 27$)	All FIV-positive cats ($n = 44$)
Breed	DSH (94%), DLH (6%)	DSH (74%), DLH (15%), Siamese X (11%)	DSH (82%), DLH (11%), Siamese X (7%)
Gender	FS (18%), MN (82%)	FS (33.3%), MN (66.6%)	FS (27%), MN (73%)
Age (years; median/range)	4/1–9	5/2–10	4.5/1–10
Age at first known positive FIV ELISA test (years; median/range)	2.3/0.2–7.8	1.6/0–4.6	1.8/0–7.8
Time from first known positive FIV ELISA test to enrolment (years; median/range)	1.3/0–3.3	3/0.1–8	2/0–8

DSH, domestic shorthair; DLH, domestic longhair; X, cross; FS, female spayed; MN, male neutered.

were observed. Bone marrow involvement was noted in all but one case. The second most common finding was nodal lymphoma, diagnosed in 5/9 cats (56%) and accompanied by splenic lymphoma in 3/9 cases (33%). One cat (M11) had a disseminated form involving the kidneys, liver, jejunum, heart, trachea and tongue. The median time since first FIV-positive ELISA test to death in cats with lymphoma was 5.3 years (range, 2.9–9 years).

Lymphoid hyperplasia and lymphoid depletion were noted concurrently in 13/16 (81%) cats. These findings were documented in various tissues, mostly in the spleen and lymph nodes, but in four cats, Peyer's patch atrophy was also diagnosed. Erythroid bone marrow hyperplasia was documented in 9/16 cats (56%), as was myeloid bone marrow hyperplasia. Multifocal lymphocytic interstitial nephritis was diagnosed in six cats (37.5%). In four cats, there were pathological signs of respiratory tract infection and pneumonia. Hypertrophic cardiomyopathy and signs of congestive heart failure were identified in the single FIV-positive cat from Group 1 that died.

None of the FIV-negative cats died during the study period.

3.4. Bodyweight

The bodyweight at the time of death for the single FIV-positive cat from Group 1 cohort that died was 15% heavier than the bodyweight recorded at enrolment (Fig. 2). The 17 FIV-positive cats from Group 2 that died lost a median of 51.3% of their bodyweight between enrolment (median 3.9 kg, range 2.27–5.54 kg) and death (median 1.9 kg, range 1.45–3.72 kg; $P < 0.0005$), over a median time span of 15 months (range 1.6–20 months). Those cats lost a median of 10.9% of their bodyweight between enrolment and 3 months before death (range −26.3% to +9.3%); median bodyweight loss in the last 3 months of life was 29.5% (range −55.4% to −8.9%).

The 16 FIV-positive cats from Group 1 that survived for the entire study period gained a median of 7.6% bodyweight over the 12 months following enrolment (enrolment, median 5.9 kg, range 3.8–8.4 kg; 12 months later 6.35 kg,

Table 3
Health status on the day of enrolment and post-mortem diagnoses for FIV-positive cats deceased during the study (Group 1, $n = 1$; Group 2, $n = 17$).

Cat #	Health status at enrolment	Post-mortem diagnosis
M3	NH	Lymphoma – retrobulbar
M5	NH	Lymphoma – bone marrow
M10	H	Lymphoma – bone marrow and lymph nodes
M11	H	Lymphoma – multicentric
M14	NH	Lymphadenomegaly; bone marrow hyperplasia
M16	NH	Pulmonary congestion and oedema with focal bacterial bronchopneumonia
M25	H	Upper and lower bacterial respiratory infection, probably secondary to viral infection
M26	NH	Chronic focal granulomatous nematode larval pneumonia; bone marrow hyperplasia
M30	NH	Lymphoid depletion; Aelurostrongylus abstrusus pneumonia; left front leg suppurative dermocellulitis; bone marrow hyperplasia, with erythroid and myeloid hyperplasia
M31	NH	Lymphoma – bone marrow
M33	NH	No post-mortem performed
M41	NH	Lymphoma – bone marrow and lymph nodes
M44	NH	Emaciation/nephritis/pancreatitis
M46	H	Lymphoma – bone marrow
M48	NH	Lymphoma – bone marrow and lymph nodes
M49	H	Lymphoma – bone marrow
M50	H	Undetermined/emaciation
C2	H	Cardiac failure, hypertrophic cardiomyopathy

C, Chicago, Group 1; M, Memphis, Group 2; H, healthy on the day of enrolment (no abnormalities detected on a physical examination); NH, not healthy on the day of enrolment (at least one abnormality detected on a physical examination).

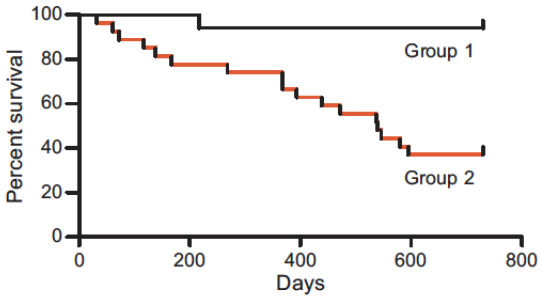

Fig. 1. Kaplan–Meier survival plots for FIV-positive cats from Group 1 (black; $n = 17$) and Group 2 (red; $n = 27$). Survival rates in Group 1 and Group 2 over the 22-month study period were 94% and 37%, respectively ($P = 0.0006$, Gehan–Breslow–Wilcoxon test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

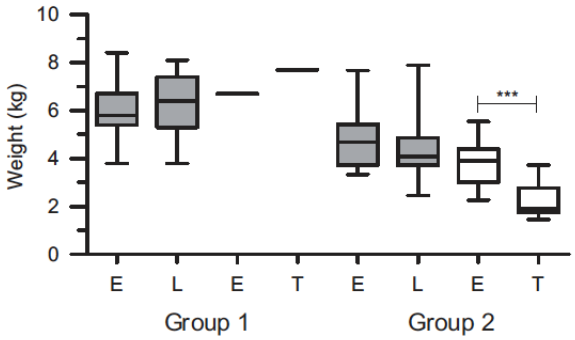

Fig. 2. Bodyweights of FIV-positive cats from Group 1 ($n = 17$) and Group 2 ($n = 27$). E, enrolment weight; L, weight at the end of the study period; T, terminal weight. Grey boxes denote cats alive at the end of the 22-month study period; White boxes denote cats dead at the end of the 22-month study period. (The central bar in the box denotes the median, while the top and bottom of the box represent the 75th and 25th centiles, respectively. The upper and lower bars represent the 95th and 5th centiles, respectively. ***$P < 0.0005$.)

range 3.9–8.1 kg; $P = 0.2089$). In contrast, the 10 FIV positive cats from Group 2 that remained alive lost a median of 12.8% of their bodyweight over the 12 months following enrolment (enrolment, median 4.68 kg, range 3.34–7.67 kg; 12 months later, median 4.08 kg, range 2.45–7.89 kg; $P = 0.2324$).

FIV-negative cats from Group 1 for which bodyweight data were available ($n = 16$) gained a median 16.8% bodyweight between enrolment (median 5.35 kg, range 4–8.5 kg) and 12 months later (median 6.25 kg, range 4.1–8 kg; $P = 0.0145$). Similarly, FIV negative cats from Memphis for which bodyweight data were available ($n = 24$) gained a median of 24.5% bodyweight between enrolment (median, 4.9 kg, range 2.7–9 kg) and 12 months later (median 6.1 kg, range 2.6–9.9 kg; $P = 0.0022$).

Available bodyweight data from Group 2 demonstrated that enrolment weights for FIV-positive cats from Group 2 that died were significantly lower than those for matched FIV-negative cats ($n = 17$; FIV-positive, median 3.9 kg, range 2.27–5.54; FIV-negative, median 5.6 kg, range 2.7–6.8 kg; $P = 0.0046$). Bodyweights were not statistically different when FIV-positive survivors from Group 2 and matching FIV-negative cats were compared at enrolment ($n = 10$; FIV-positive, median 5.12 kg, range 3.34–7.68 kg; FIV-negative median 5.8 kg, range 4.6–9.0 kg; $P = 0.1641$) and 12 months later (FIV-positive, median 4.63 kg, range 3.63–8.27 kg; FIV-negative, median 6.2 kg, range 4.9–9.9 kg; $P = 0.0977$).

3.5. CD4+ T cell counts and CD4:CD8 ratio

Fig. 3 illustrates the CD4:CD8 ratios obtained over 12 months for the FIV-positive cats from Group 2 with reference values acquired from FIV-negative cats classified as healthy from the same geographic location. The difference between FIV-positive (median 0.89, range 0.19–2.24) and FIV-negative cats (median 1.71, range 1.03–2.62) was statistically significant at the time of enrolment ($P < 0.0001$) and 12 months later (medians 0.69, range 0.11–1.54 and 1.48, range 0.88–2.77 for FIV-positive and FIV-negative cats, respectively; $P < 0.0001$). The CD4:CD8 ratio in surviving FIV-positive cats was maintained over 18 months at a consistently low level (median values: 0.89, 0.73, 0.69, and 0.81 for each time point).

CD4:CD8 ratio at the time of enrolment ($P < 0.0005$) was lower in the FIV-positive cats from Group 1 (median 0.77, range 0.08–1.27) than in matched FIV-negative cats (median 2.18, range 1.23–6.42; Fig. 3). CD4:CD8 ratios in the FIV-positive cats from Group 1 were maintained at a consistently low level over the 12 months following enrolment (median 0.65, range 0.2–2.55) compared to age- and sex-matched FIV-negative cats (median 1.8, range 1.4–3.93; $P = 0.0054$). The CD4:CD8 ratios at the time of enrolment in the FIV-positive cats from Group 1 were lower (median 0.77, range 0.08–1.27) than those of

Table 4
Organs with neoplastic lymphocyte infiltrates among FIV-positive cats diagnosed with lymphoma at post-mortem.

Cat #	Bone marrow	Lymph node	Spleen	Kidney	Liver	Jejunum	Tongue	Trachea	Heart	Eye
M3										+
M5	+	+	+							
M10	+	+								
M11	+	+	+	+	+	+	+	+	+	
M31	+									
M41	+	+	+							
M46	+									
M48	+	+								
M49	+									

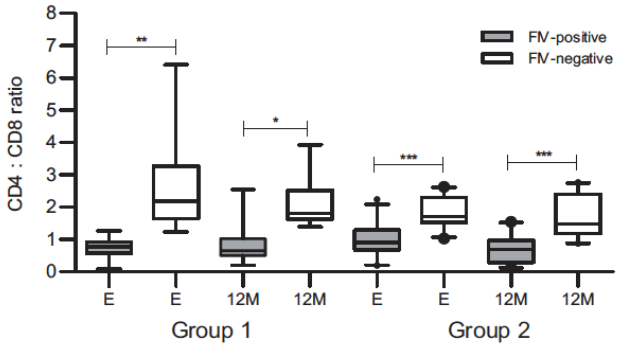

Fig. 3. CD4:CD8 ratios for FIV-positive cats from Group 1 and Group 2 (in grey) and age- and sex-matched FIV-negative cats from the same geographic location (in white). For Group 1, data represent 17 FIV-positive cats and 17 age- and sex-matched FIV-negative cats at enrolment (E) and 16 surviving FIV-positive and 16 FIV-negative cats at 12 months after enrolment (12M). In Group 2 at enrolment, data were collected from 27 FIV-positive cats and 27 FIV-negative cats; 12 months after enrolment, data were collected from 21 surviving FIV-positive cats and 21 FIV-negative cats. (The central bar in the box denotes the median, while the top and bottom of the box represent the 75th and 25th centiles, respectively. The upper and lower bars represent the 95th and 5th centiles, respectively. $^{*}P < 0.01$; $^{**}P < 0.001$; $^{***}P < 0.0001$.)

the FIV-positive cats from Group 2 (median 0.89, range 0.19–2.23; Fig. 3).

There were no statistically significant differences when the CD4:CD8 ratio was compared between cats classified as healthy and not healthy at enrolment at either group, or for all FIV-positive cats as a single group (Group 1, $P = 0.97$; Group 2, $P = 0.84$; all FIV-positive cats, $P = 0.99$; Fig. 4).

To determine the kinetics of the CD4:CD8 ratio inversion over the course of infection, we compared absolute CD4+ and CD8+ T lymphocyte numbers from 17 cats from Group 2 that died during the study (Fig. 5). The decrease in CD4+ T cell count was statistically significant when enrolment results (median 0.41 K/μL, range 0.13–1.38 K/μL) were compared with terminal results (median 0.18 K/μL, range 0.04–1.32 K/μL; $P = 0.0017$), but this was not the case for the decrease in

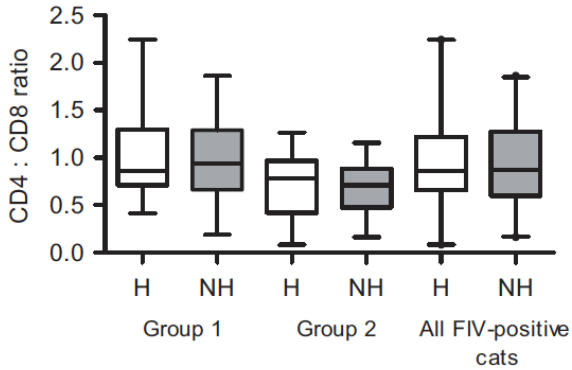

Fig. 4. CD4:CD8 ratio at enrolment for FIV-positive cats from Group 1 ($n = 17$), Group 2 ($n = 27$) and all FIV-positive cats ($n = 44$). Cats were classified as healthy (H) or not healthy (NH) based on physical examination findings at the time of enrolment. (The central bar in the box denotes the median, while the top and bottom of the box represent the 75th and 25th centiles, respectively. The upper and lower bars represent the 95th and 5th centiles, respectively.)

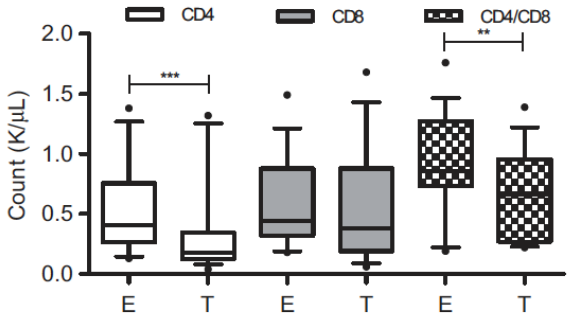

Fig. 5. CD4+ and CD8+ T cell count and CD4:CD8 ratio at enrolment (E) and terminally (T) from the FIV-positive cats from Group 2 that died during the study ($n = 17$). (The central bar in the box denotes the median, while the top and bottom of the box represent the 75th and 25th centiles, respectively. The upper and lower bars represent the 95th and 5th centiles, respectively. $^{**}P = 0.0034$; $^{***}P = 0.0017$.)

CD8+ T cell count from enrolment (median 0.44 K/μL, range 0.18–1.49 K/μL) to terminal disease (median 0.38 K/μL, range 0.06–1.68 K/μL; $P = 0.35$). Therefore, the decrease in the CD4:CD8 ratio was attributed to a reduction in the CD4+ T cell count. Further analysis of total lymphocyte counts in deceased cats showed that the decrease in absolute lymphocyte numbers between enrolment (median 1.73 K/μL, range 1.15–4.03 K/μL) and terminal disease (median 1.25 K/μL, range 0.25–3.55 K/μL) reflected not only the loss of CD4+ T cells, but also depletion of CD21+ B cells (enrolment, median CD21+ B cell count 0.26 K/μL, range 0.12–0.89 K/μL; terminal specimens, median CD21+ B cell count 0.17 K/μL, range 0.04–0.37 K/μL; $P = 0.0061$).

3.6. FIV load (FIV genomes/mL blood)

The FIV load at enrolment was significantly lower in Group 1 (median, 741 genomes/mL blood; range 43–18,796 genomes/mL blood) than Group 2 (median, 50,003 genomes/mL blood; range, 2540–3,792,000 genomes/mL blood; $P < 0.0001$; Fig. 6). There were no statistically

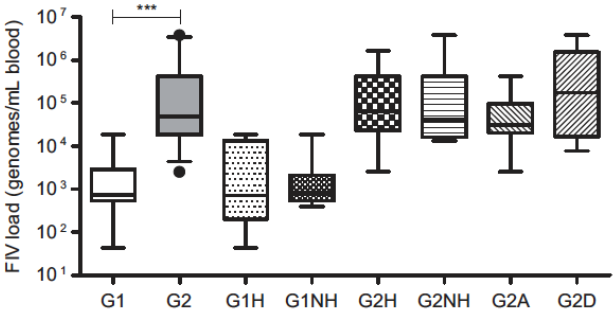

Fig. 6. FIV load at the time of enrolment (genomes/mL blood). Statistical comparisons were made between pairs of classifications across the graph. G1, Group 1 ($n = 15$); G2, Group 2 ($n = 26$); H, healthy on the day of enrolment (no abnormalities detected on a physical examination; Group 1, $n = 8$, Group 2, $n = 11$); NH, not healthy on the day of enrolment (at least one abnormality detected on a physical examination; Group 1, $n = 7$, Group 2, $n = 15$); A, alive at the end of the 22-month study period (Group 2, $n = 9$); D, cats dead at the end of the 22-month study period (Group 2, $n = 17$). $^{***}P < 0.0001$.

significant differences in FIV load at enrolment when healthy and not healthy cats were compared in Group 1 (healthy, $n = 8$, median 717 genomes/mL blood; range, 43–18,796 genomes/mL blood; not healthy, $n = 7$, median 767 genomes/mL blood; range, 395–18,796 genomes/mL blood; $P > 0.05$) or Group 2 (healthy, $n = 11$, median 64,843 genomes/mL blood; range, 2540–1,667,000 genomes/mL blood; not healthy, $n = 15$, median 41,387 genomes/mL blood; range, 13,423–3,792,000 genomes/mL blood; $P > 0.05$). In Group 2, there were no significant differences in FIV load at enrolment when the following comparisons were made: cats still alive vs. cats that had died by the end of the study period (alive, $n = 9$, median 31,395 genomes/mL blood; range, 2540–41,8671 genomes/mL blood; dead, $n = 17$, median 176,551 genomes/mL blood; range, 7724–3,792,000 genomes/mL blood; $P > 0.05$); cats that had died by the end of the study period and were classified at enrolment as either healthy or not healthy (healthy, $n = 6$, median 241,757 genomes/mL blood, range, 7724–1,667,000 genomes/mL blood; not healthy, $n = 11$, median 176,551 genomes/mL blood; range, 13,423–3,792,000 genomes/mL blood; $P > 0.05$); and cats that were still alive by the end of the study period and were classified at enrolment as either healthy or not healthy (healthy, $n = 5$, median 56,867 genomes/mL blood, range, 2540–418,671 genomes/mL blood; not healthy, $n = 4$, median 27,771 genomes/mL blood, range 16,176–43,138 genomes/mL blood; $P > 0.05$).

Statistical correlations between FIV load and CD4+ T cells, CD8+ T cells and the CD4:CD8 ratio were investigated for each group. There were statistically significant, or close to significant, negative correlations between the CD4:CD8 ratio and FIV load in Group 1 ($r = -0.5022$; $P = 0.0564$) and in Group 2 ($r = -0.5479$; $P = 0.0038$; Fig. 7).

3.7. Phylogenetic classification of FIV envelope genes

Full-length viral envelope (*env*) gene sequences from FIV-positive cats were examined ($n = 355$). In Group 1, clade B *env* viruses and clade A/B recombinant *env* viruses each accounted for 50% of infections. In Group 2, 69% of cats were infected with viruses bearing clade B *env*, while 31% of cats were infected with recombinant *env* viruses (Supplementary Table 3).

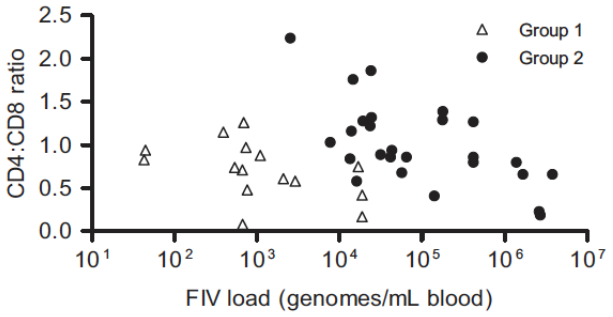

Fig. 7. Correlation between FIV load (FIV genomes/mL blood) and CD4:CD8 ratio for FIV-positive cats from Group 1 ($n = 15$; $r = -0.5022$, $P = 0.0564$) and Group 2 ($n = 26$; $r = -0.5479$, $P = 0.0038$).

4. Discussion

Here we describe contrasting clinical outcomes of naturally acquired FIV infection, demonstrating significant differences between cats enrolled from two separate cohorts. The relationship between FIV infection and clinical disease is unclear, with some studies reporting that infected cats are at increased risk of morbidity and mortality (Muirden, 2002), while others report no such association (Akhtardanesh et al., 2010; Liem et al., 2013). Considering all 44 FIV-positive cats, there was no relationship between mortality and morbidity and FIV infection; however, this was not the case when the two cohorts were examined separately. Our observations in Group 2 indicated that living conditions might have played a role in the more rapid disease onset. In contrast, cats from Group 1 were generally housed in single cat households and remained alive and in relatively good health over the study period. Therefore, it appears that FIV infection is more likely to progress in cats kept in crowded shelter conditions compared to those living in spacious environments. As a corollary, our results suggest that the FIV-positive cats in our study that lived in single or dual cat households remained free of clinical signs of illness over the study period; this can most likely be attributed to lower risks of exposure to opportunistic pathogens and reduced levels of environmental stress.

FIV infected cats are at greater risk of opportunistic infections and neoplasia (Hartmann, 2012). Regular assessment of their health status is essential and, according to European Advisory Board on Cat Diseases guidelines (Hosie et al., 2009), health checks are recommended every 6 months. The short time to disease progression leading to the deaths of 63% of cats in Group 2 within the 22-month follow-up period was unexpectedly high, especially in light of previously published studies that were unable to demonstrate a difference in lifespan between FIV-positive and FIV-negative cats (Addie et al., 2000; Ravi et al., 2010). The health status of cats from Group 1 was similar to that of cats in a household in which FIV infection did not reduce life expectancy over a period of 2 years (Addie et al., 2000). There are several clinical abnormalities associated with FIV infection, with gingivostomatitis being the most common. Although one study (Quimby et al., 2008) did not observe an association between FIV infection and gingivostomatitis, the high prevalence observed here was in agreement with previous reports (Bandecchi et al., 1992; Hofmann-Lehmann et al., 1995; Hosie et al., 2009; Knotek et al., 1999). As gingivostomatitis is rare in SPF cats experimentally infected with FIV, co-infections with other infectious agents, such as feline calicivirus, might have contributed to this syndrome in naturally infected cats (Reubel et al., 1994; Tenorio et al., 1991). It is perhaps relevant that severe acute on chronic upper respiratory tract disease was diagnosed in four cats from Group 2 at post-mortem. Other abnormalities previously attributed to FIV-induced dysregulation and impairment of immune surveillance include dermatitis, ocular disease, renal insufficiency, lower urinary tract infections and other opportunistic infections (Barlough et al., 1991; Reche et al., 2010). Although neurological

abnormalities have been reported in natural and experimental, acute and chronic FIV infections (Abramo et al., 1995; Dow et al., 1990; Podell et al., 1997, 1999), neither behavioural nor motor abnormalities were observed in our study population.

The mortality rate of 63% in the FIV-positive cats from Group 2 was markedly higher than the FIV-positive cats from Group 1, in which only one cat died during the study period (6%). Post-mortem examinations identified several pathological changes in the FIV-infected cats. Although FIV infection is generally associated with immunosuppression, immune hyperstimulation is evident during the early stages of infection, including B cell activation, manifested as polyclonal hypergammaglobulinemia (Hopper et al., 1989; Takano et al., 2012), expansion of CD8+ T cells with an activated phenotype and subsequent CD8+ T lymphocytosis (Beatty et al., 1996; Willett et al., 1993). Lymph node follicular hyperplasia and dysplasia was documented post-mortem in this study, confirming the findings of other studies of acute and terminal stage disease (Matsumura et al., 1993; Shelton et al., 1989; Yamamoto et al., 1988). In addition, reactive changes in the bone marrow, observed in 56% of cats in this study, have been documented previously in HIV and FIV infections (Bain et al., 2009; Breuer et al., 1998; Geller et al., 1985).

Lymphoma was the most common condition diagnosed at post-mortem in Group 2 (9/16 cats; 56%), which raises questions regarding potential interactions between virus, host, environment, and other factors that could have resulted in a predisposition to neoplasia. FIV strain variability was examined, since the Memphis FIV strains (Group 2) might have had greater oncogenic potential than the strains infecting the Chicago cohort (Group 1). However, a comparison of full-length *env* gene and long terminal repeat sequences revealed no significant differences (unpublished observation). It is possible that the local cluster of lymphoma in Group 2 was associated with another infectious agent, such as FeLV, but this seemed unlikely since FIV/FeLV Snap test (IDEXX) results were negative for FeLV antigen. However, we cannot exclude the possibility of regressive FeLV infection (Hofmann-Lehmann et al., 2001), since PCR testing to detect integrated proviral DNA in bone marrow was not performed. Breed was unlikely to play a role, as the cats from Group 2 diagnosed with lymphoma and those from Group 1 were all classified as domestic shorthairs and came from a variety of sources within their geographic area. The shelter environment in which the cats from Group 2 lived was likely to play a role in the development of the various clinical manifestations of FIV, although exposure to potential carcinogens cannot be ruled out.

Marked weight loss between enrolment and terminal measurement was evident among the cats that died over the study period, whereas healthy FIV-infected cats maintained relatively stable bodyweights (Fig. 2). Although monitoring bodyweight regularly can be a valuable tool in the care of FIV-positive cats and appeared to have some prognostic value in our study of FIV-positive cats, declining bodyweight is a relatively non-specific finding, as it is not unusual to observe weight loss during the progression of various other diseases.

The hallmark of FIV infection is an inverted CD4:CD8 ratio, which, as demonstrated here and elsewhere (Hoffmann-Fezer et al., 1992; Novotney et al., 1990; Tompkins et al., 1991), is largely the consequence of a loss of CD4+ T cells. Here we provide further evidence that FIV-positive cats had significantly lower CD4:CD8 ratios than those for FIV-negative cats over the 22-month study period in each of the cohorts (Fig. 3). We also observed a statistically significant decline in the CD4:CD8 ratio between the time of enrolment and the terminal sampling in those cats that died over the study period (Fig. 5). However, there was not a significant difference in median CD4:CD8 ratio at enrolment when cats classified as either healthy or not healthy were compared (Fig. 4). Both groups of cats had relatively low CD4:CD8 ratios with median values less than 1, regardless of their health status. Indeed, the cat that had the lowest CD4+ T lymphocyte count (0.09 K/μL) and the lowest CD4:CD8 ratio (0.08) at enrolment was classified as healthy and is still alive. Similar observations have been reported previously in FIV (Novotney et al., 1990) and HIV infections (Levy, 1988), where seropositive individuals remained asymptomatic despite significant loss of CD4+ T lymphocytes. The variability in CD4:CD8 ratios among healthy and not healthy cats in this study population also limited their utility in the prediction of disease progression.

Extrapolating from HIV infection, the plasma viral load has been postulated as a potential prognostic indicator for FIV infection. A study focusing on disease progression in SPF cats experimentally infected with two strains of FIV of different virulence reported a correlation between disease progression and increased plasma viral load (Diehl and Hoover, 1995). Similarly, the relationship between plasma viral load, disease progression and survival time was found in a study of naturally infected cats in Japan (Goto et al., 2002). In the present study, the median FIV load at the time of enrolment from Group 2 was 67-fold higher than that from Group 1 ($P < 0.0001$) and there was a marked difference in health status and mortality rate between groups over the following 22 months. It is not possible to discern whether an increased viral load was a cause or a consequence of poor health status, since that would have required knowledge of viral load kinetics and changes in health status from the time of initial infection in the FIV-infected 'healthy' and 'not healthy' cats; in this prospective study the data were collected from the time of enrolment. However, statistically significant differences in FIV load between cats from Group 2 classified as 'healthy' and 'not healthy' and cats that remained alive or died during the study were not apparent. Observation of these parameters over longer periods of time, preferably with larger group sizes, would be required to fully validate their utility in predicting disease progression.

It has been hypothesized that FIV clade B viruses are evolutionarily older and more host-adapted, hence less pathogenic, than those of clade A (Sodora et al., 1994). One possible explanation for the high morbidity and mortality in Group 2 is that those cats were infected with highly virulent strains of FIV. However, this hypothesis was not supported by the phylogenetic analyses performed, as cats

from both groups were infected with comparable numbers of clade B and recombinant A/B viruses. Furthermore, sequences isolated from serial 6-monthly blood collections demonstrated that highly monophyletic groups were formed in 96% of the cats from Group 2, suggesting that viral isolates were not transmitted between cats despite unrestricted cohabitation (Bęczkowski et al., 2014). Although we cannot exclude the possibility that cats from Memphis were infected with more virulent FIV isolates, based on thorough phylogenetic analyses of full-length *env* sequences, this seems unlikely.

A limitation of this study is the restriction of enrolments to two distinct geographic areas; the inclusion of cats from a wider range of locations could have increased the variability in virulence of the wild type FIVs examined and their possible contribution to the onset of disease. Additionally, for the purposes of this study of naturally infected cats either adopted from shelters or rescued, it was necessary to estimate the ages of the cats and to use the date that the cat first tested FIV-positive since the precise dates of infection were not known. It has been reported previously that age at time of first exposure to FIV can influence the course of infection (George et al., 1993) and can determine the outcome of disease (Akhtardanesh et al., 2010). From a biological and behavioural perspective, the age of approximately 1–2 years old at the time of first diagnosis reported in this study is consistent with young cats engaging in territorial fights, especially if they are entire. The cats in our study were originally adopted from shelters and many were entire and had outdoor access at the time of shelter intake, both known lifestyle risk factors for FIV (Levy et al., 2006). In our study population, infection was more common in male cats, confirming the results of previous studies (Grindem et al., 1989; Hosie et al., 1989; Ishida et al., 1989; Levy et al., 2006; Yamamoto et al., 1989). Additionally, FIV status and multi-cat housing status could have affected the health of the cats in our study; it was not possible to discern whether they acted independently or as cofactors. FIV-negative cats matched to enrolled FIV-positive cats on housing status, as well as age and sex, were not available during the study enrolment period. While an extra layer of matching based on housing status would have been ideal, it was not achievable in this real-world study.

Based on the relatively large number of cats enrolled and the striking clinical differences observed between the two cohorts, we conclude that keeping FIV-infected cats in overcrowded conditions can have a significant impact on the risk of disease progression, particularly in cats which already have their immune systems compromised by FIV infection. In contrast, FIV-positive cats remained in relatively good health when living in stable, single cat households. Clinical signs of disease were not observed at 6-monthly physical examinations over a 24-month period of the cats from Group 1 that were housed in single cat households. The average duration of the 'asymptomatic' phase of infection remains unknown and could only be estimated following observations over a longer period. In this study, the selection of study participants from different locations and housing conditions minimized potential selection bias. It is apparent that the conclusions of this study would have been very different indeed had efforts been focused on only one of the cohorts presented herein.

Conflict of interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.12.023.

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FIV FAQ Sheet

What is FIV?
FIV is feline immunodeficiency virus. It's a virus that lives in the blood and saliva of cats.

Is FIV contagious to humans?
No. This virus can only survive inside of the feline body.

Is it contagious to any animals?
Yes, it is contagious to felines only. It is not contagious to dogs or other species.

How do cats get FIV?
It can only be spread through sex and deep penetrating bite wounds only. It is not spread by grooming, eating after each other, sharing a litter box, etc.

Do FIV cats need medication?
Nope, you treat them just like any other cat. Regular veterinary care is important to all cats, including those with FIV.

What, if anything, does FIV do to a cat?
It can cause a weakened immune system, but typically does not. Most FIV cats live long normal healthy lives; statistically there is no difference in lifespan. Sometimes they can get gingivitis, and if they do get sick they may get sicker faster than a negative cat. So catching illnesses early in an FIV cat is important as well as taking them in for annual check ups. But lots of people own FIV+ cats and don't even know it because they never got them tested. FIV was discovered in 1986. If you owned cats prior to 1986, they may have had an FIV+ cat and you never knew it!

Will FIV live in my house or carpet?
No. The FIV virus dies very rapidly in the environment. You don't have to worry about carrying it on your clothes. You can safely house FIV negative cats in the same place you previously housed FIV positive cats, and can even house non-aggressive, altered positive and negative cats together, without significant risk of transmission.

Will FIV get my personal cats sick?
Since FIV is only spread through deep bite wounds or sex, the standard answer is "no". All APA! cats are spayed/neutered so there is no risk of spreading through sex (even if cats "mount" each other for dominance reasons they will not actually penetrate). Most cats, if they don't get along, don't actually bite each other hard enough to draw blood and spread FIV – typically they hiss, scratch, or maybe give warning bites, but it is very rare for neutered house cats to bite like that. If your personal cat and your newly adopted FIV cat are fighting that severely, FIV isn't your biggest concern! We house our FIV+ cats with FIV- cats, so long as they get along, and have never had FIV transmission. If we have an FIV+ cat we know does not get along with other cats we will let you know prior to adoption.

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FIV Terminology Protocol: Words Matter

We hear it all the time: "FIV…that's feline AIDS, right?" No.

At APA!, we do not refer to FIV (Feline Immunodeficiency Virus) as "AIDS" because it is not scientifically accurate, it perpetuates dangerous myths which prevent FIV+ cats from being adopted, and it contributes to their needless deaths in shelters around the country. It is our job to advocate for cats, educate visitors, and dispel outdated shelter policies which harm cats.

For these reasons, when we are faced with the term "Feline AIDS", all cat program staff is to educate the speaker on why APA! does not use that term, and why.

The FAQ

A positive test for FIV doesn't even mean a cat has FIV, let alone AIDS (acquired immunodeficiency syndrome). Current tests for FIV test for antibodies to FIV, which means that if a cat has previously been vaccinated for FIV, it will test positive for the remainder of the cat's life. There is no way to tell the difference between an infected cat and a vaccinated cat.

Even if the cat does have FIV, there are different stages of the virus:

- **Stage 1**
  - After initial infection with FIV, a cat may, but usually does not, appear mildly ill. The cat's body fights the virus, and they may progress to stage 2.
- **Stage 2**
  - In phase 2, cats are usually completely healthy, showing no signs of the virus at all. This stage can, and does, last for many years, if not forever!
- **Stage 3**
  - This is the stage of the virus where it has weakened the immune system and the cat becomes more susceptible to other illnesses. However, this stage usually takes many years to develop, and it may not even develop at all!
  - Stage 3 is the closest resemblance to "AIDS" and remember, not all FIV+ cats even acquire this stage!

FIV is not the same as Feline AIDS. Feline AIDS refers to the final, terminal stages of disease that can be caused by FIV, but not all cats with FIV develop AIDS.

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Transmission of feline immunodeficiency virus (FIV) among cohabiting cats in two cat rescue shelters

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ABSTRACT

Conflicting accounts have been published in the veterinary literature regarding transmission of feline immunodeficiency virus (FIV) between cohabiting cats in mixed households, and the mechanics of possible casual transmission, if it occurs, are poorly understood. Similarly, there are conflicting reports of vertical transmission of FIV. The aim of the present study was to document the FIV serological status of cats taken into two rescue shelters. At rescue shelter 1 (Rescue 1), cats cohabited in a multi-cat household of FIV-negative and naturally-infected, FIV-positive cats. A study was performed that combined a retrospective review of records of FIV serological status at intake (Test 1) and prospective FIV serological testing (Tests 2 and 3). Retrospective records were analyzed at rescue shelter 2 (Rescue 2), where FIV-positive queens with litters of nursing kittens were taken into the shelter, before being rehomed. FIV serology was performed on all kittens after weaning.

Initial test results (Test 1) for 138 cohabiting cats from Rescue 1 showed that there were 130 FIV-negative cats and eight FIV-positive cats (six male neutered and two female spayed). A second test (Test 2), performed in 45 of the FIV-negative and five of the FIV-positive cats at median 28 months after Test 1 (range, 1 month to 8.8 years) showed that results were unchanged. Similarly, a third test (Test 3), performed in four of the original FeLV-negative cats and one remaining FIV-positive cat at median 38 months after Test 1 (range, 4 months to 4 years), also showed that results were unchanged. These results show a lack of evidence of FIV transmission, despite years of exposure to naturally-infected, FIV-positive cats in a mixed household. At Rescue 2, records were available from five FIV-positive queens with 19 kittens. All 19 kittens tested FIV-negative, suggesting that vertical transmission had not occurred.

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Introduction

Feline immunodeficiency virus (FIV) is a retrovirus of the genus *Lentivirus*, which is endemic in cat populations worldwide. While the main mode of transmission is via bite wounds, vertical or pediatric transmission (in utero, intrapartum, milk/colostral) and transmission between cats in stable households, is much less common (Shelton et al., 1989). Under laboratory conditions, FIV is also infectious via parenteral routes, such as intravenous, intraperitoneal, intradermal and subcutaneous (Burkhard and Dean, 2003). Mucosal transmission of experimental infections has also resulted from virus inoculation into the nasal cavity, mouth, vagina and rectum, but transmission of FIV across mucosal surfaces is relatively ineffective compared to human immunodeficiency virus (HIV; Bishop et al., 1996). Previously published studies of closed 'mixed households' of cohabiting FIV-negative and FIV-positive cats have reported variable infection transmission rates (Table 1), but the prevalence of episodes of inter-cat aggression was usually not quantified and the observation periods were often relatively short.

There have been a number of observational studies of vertical transmission of naturally-acquired FIV infections (Yamamoto et al., 1988; Ueland and Nesse, 1992; Medeiros et al., 2012) and experimental studies using FIV to develop models of vertical transmission of HIV (Yamamoto et al., 1988; Sellon et al., 1994; O'Niel et al., 1996; Rogers and Hoover, 1998). The study by Ueland and Nesse (1992) was unable to demonstrate vertical or horizontal transmission of FIV among 25 adult cats (6 FIV-positive, 19 FIV-negative) and 48 kittens (30 of which were born to FIV-positive queens) in a closed breeding colony over a 9 month period. A later study, using molecular methods and serology, demonstrated vertical transmission of naturally-acquired infection from one queen to one kitten (Medeiros et al., 2012). Chronically-infected queens in one experimental model transmitted the virus to approximately half of their offspring via late in utero, intrapartum and milk-borne routes (O'Niel et al., 1996), and approximately 60% of full term fetuses were infected in another chronic infection model (Rogers and Hoover, 1998). Milk-borne infection was demonstrated at a similar rate (62.5%) in kittens born to acutely-infected queens (Sellon et al., 1994). Another study dem-

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Table 1
Published studies of closed 'mixed' populations of FIV-negative and FIV-positive cats.

Reference	FIV-negative (*n*)	Originally FIV-positive (*n*)	Originally FIV-negative cats that became infected (*n*)	Laboratory/ home	Observation period
Yamamoto et al., 1988	14	18	0	Laboratory	4–14 months
Shelton et al., 1989	31	16	0	Home	Median of 2 years
Shelton et al., 1990	68	5	0	Home	3.5 years
Dandekar et al., 1992	20	NR	1[a]	Laboratory	2–4 years
Addie et al., 2000	17	9	6	Home	10 years

NR, not recorded.
[a] In this study, 10/19 cats that remained serologically FIV-negative at the end of the study period tested FIV-positive using PCR.

onstrated that maternally-derived anti-FIV antibodies from either vaccinated or infected queens could prevent infection in neonatal kittens inoculated intraperitoneally (Pu et al., 1995).

The aims of the present study were to investigate horizontal transmission of naturally-acquired FIV between cats in a mixed, multi-cat household and to investigate viral transmission from naturally-infected FIV-positive queens to their kittens.

Materials and methods

Study design

Information was collected from two cat-only rescue shelters (designated Rescue 1 and Rescue 2). At Rescue 1, a retrospective review of records of FIV serological status at intake (Test 1, $n = 138$) was performed for all cats that had entered the shelter since its inception in October 2003. Some cats had also received a second and a third FIV serological test (Test 2, $n = 16$; Test 3, $n = 2$). Prospective FIV serological testing was performed on all cats that were still present at the shelter on 16 November 2011 (Test 2, $n = 34$; Test 3, $n = 3$). Additional information was also obtained regarding housing status (indoor-only vs. access to outdoors) and any history of inter-cat aggression for the duration of each cat's stay at Rescue 1. At Rescue 2, records were reviewed for information relating to FIV-positive pregnant and nursing queens and their kittens that had been presented to the shelter.

Animals and FIV testing

Approval for this study was granted by the Purdue University Animal Care and Use Committee (Protocol number 1201000568; 9 February, 2010).

For Rescue 1, cats cohabited in a single household, sharing litter pans, food/water dishes and bedding. This was a privately owned rescue facility based in a domestic home, run by single caregiver. Cats were sourced from local partner shelters after they were spayed/neutered and they left the rescue facility when adopted. All cats were housed indoors only, except for one cat (Cat A; FIV-positive at Test 1), which was allowed occasional outdoor access. None of the cats were known to have any history of vaccination against FIV. Records were available from initial serological testing (Test 1) for feline leukemia virus (FeLV) and FIV (SNAP FIV/FeLV Combo Test, IDEXX Laboratories) from 138 cats at the rescue facility. All 138 cats in the study were FIV tested on the day they entered the shelter, except for one cat that was determined to be FIV-positive 17 months before shelter entry and four FIV-negative cats that tested FIV-negative at 4 months ($n = 2$), 11 months ($n = 1$) and 18 months ($n = 1$) before shelter entry. These cats were still FIV-negative at 14 months ($n = 2$) and 2 months ($n = 2$) after shelter entry, respectively. Veterinarians and staff at the shelters where the cats were originally sourced performed Test 1 at the time of spay/neuter, before the cats arrived at the rescue.

After adoptions from Rescue 1, 50 of the original 138 cats that underwent Test 1 still remained at the rescue facility and these underwent a second test (Test 2; IDEXX SNAP FIV/FeLV Combo Test) a median of 28 months after Test 1 (range, 1 month to 8 years 10 months; interquartile range 15 months to 3 years 9 months). The rescue facility caregiver performed Test 2 on 13/50 cats and a certified veterinary technician tested the remaining 37 cats. PCR testing for FIV (RealPCR Test for FIV, IDEXX Laboratories) was also performed on 33/50 cats, using the same blood collected for Test 2. Personnel performing the follow-up tests were aware of previous test results.

A third test (Test 3; IDEXX FIV/FeLV SNAP Combo Test) was performed on 5/50 cats that underwent Test 2. These five cats had been continuously housed at the rescue facility since entry and initial testing. Test 3 was undertaken a median of 3 months after Test 2 (range, 1–3 years 9 months; interquartile range 1 month – 2 years 6 months). The same veterinary technician who performed Test 2 performed Test 3.

Rescue 2 was a privately-owned, feline-only rescue facility, which accepted stray and owner-relinquished cats. Cats were assessed by a resident veterinarian and serological testing (IDEXX FIV/FeLV SNAP Combo Test) was performed on entry. Any heavily pregnant FIV-positive queens were transferred to foster homes, once initial assessment and testing had been completed. While in foster care, queens and their litters did not have access to any other FIV-positive cats. Queens were returned to the rescue facility for ovariohysterectomy after their kittens had been weaned at approximately 8 weeks of age. Kittens were tested for FIV/FeLV (IDEXX FIV/FeLV SNAP Combo Test) after weaning. Queens and kittens then offered for adoption if deemed healthy by the rescue veterinarian. Vaccination against FIV was not performed at the rescue center, or while the cats were in foster care.

Data analysis

Month-by-month infection pressure was calculated by tracking the number of FIV-positive and FIV-negative cats cohabiting each month since the first FIV-positive cat entered the shelter (March 2008). Calculations for FIV-negative cats that were adopted during the study period assumed that the adoption date (rather than the most recent test date) was the end date of the calculation, so that complete residence time in the population was used for the calculation. Descriptive statistics were calculated using commercially available software (GraphPad Prism 5 for Mac OS X).

Results

Rescue 1

Information on cats recruited into the study and results of FIV testing are presented in Table 2. For each cat tested, the FIV serological status remained the same at all time-points assessed. It was reported that one of the FIV-positive cats (Cat A; male neutered [MN] domestic shorthair [DSH], aged approximately 2 years at Test 1; residing in the household for 2 years and 4 months during the study period) was frequently aggressive toward other cats, but skin wounds were not noted on either cat after episodes of aggression. Another two FIV-positive cats (Cat B, MN DSH, aged approximately 2.5 years at Test 1, resided in the household for 1 year and 4 months during the study period; Cat C, female spayed [FS] DSH, aged 5 months at Test 1, resided in the household for 3 years and 8 months during study period) habitually groomed other cohabiting cats.

At Test 1 ($n = 138$) and Test 3 ($n = 5$), all cats tested FeLV-negative. At Test 2 ($n = 50$), one cat (Cat D; MN DSH, aged approximately 8 years at Test 2) tested FeLV-positive and FIV-negative. Cat D was

Table 2
Descriptive information for cats tested for FIV/FeLV (IDEXX FIV/FeLV SNAP Combo Test) at Rescue 1.

	FIV-negative cats	FIV-positive cats
Test 1 (*n*)	130	8
Test 1 – Median age[a] (range)	4 months (2 months – 13 years)	28 months (5 months – 11 years)
Test 1 – Gender[b]	71 MN, 59 FS	6 MN, 2 FS
Test 2 (*n*)[c]	45	5
Test 2 – Median age[a] (range)	4 months (2 months – 8 years)	23 months (5 – 31 months)
Test 2 – Gender[b]	25 MN, 20 FS	4 MN, 1 FS
Test 3 – *n*[c]	4	1
Test 3 – Median age[a] (range)	30 months (7 months – 4 years)	4 years
Test 3 – Gender[b]	2 MN, 2 FS	1 FS

MN, male neuter; FS, female spayed.
[a] The veterinarians and staff at the source shelters from which the cats were obtained estimated cat age at the time of spay/neuter.
[b] Gender refers to gender status at the time of admission to the private rescue facility.
[c] Cats tested at Test 2 were a subset of cats tested at Test 1. Cats tested at Test 3 were a subset of cats tested at Test 2.

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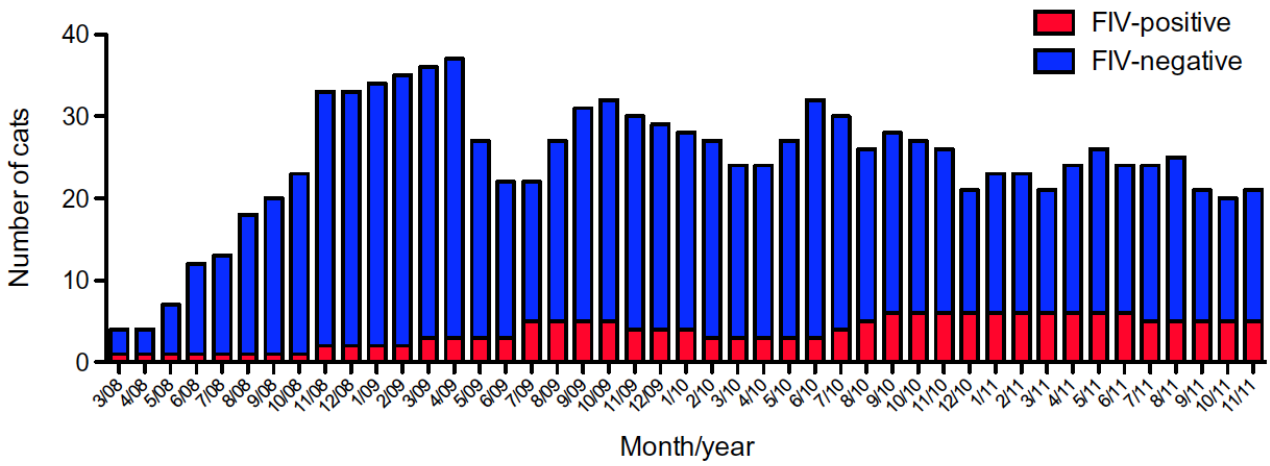

Fig. 1. Number of FIV-positive and FIV-negative cats cohabiting at Rescue 1 month by month (March 2008–November 2011).

the only cat that tested FeLV-positive at Rescue 1 during the study period. This cat had left the household after Test 1 was performed and was subsequently retested (Test 2) before being considered for readmission to the household, but because of the FeLV-positive result, the cat was not readmitted and died 2 months later.

Results of FIV testing by PCR, performed on whole blood samples collected for Test 2 in 33/50 cats showed that both ELISA and PCR results were negative in 29/33 cats; both ELISA and PCR results were positive in 3/33 cats, and the remaining cat tested ELISA-positive but PCR-negative.

Infection pressure results are presented in Figs. 1 and 2. The ratio of FIV-negative to FIV-positive cats cohabiting at Rescue 1 month by month (March 2008–November 2011) ranged from 2.5–22.0, while the total number of cats ranged from 4 to 37 over the study period.

Rescue 2

Serological test results from five queens and their 19 kittens from Rescue 2 are presented in Table 3. All five queens were presented as strays, so their previous vaccination history was unknown. Although demonstrated to be FIV positive by serology, FIV PCR or other FIV confirmatory testing was not performed.

Discussion

In this study of cats cohabiting in a mixed household over a period of months to years, despite mutual grooming, mild aggression, shared food bowls, litter boxes, bedding etc. there was no evidence of transmission of infection from FIV-positive to FIV-negative cats. Additionally, at a second rescue shelter, serological testing did not demonstrate any evidence of vertical transmission of FIV from naturally-infected mothers to their kittens. The number of infectious units transmitted (Kusuhara et al., 2005) and the viral phenotype (Burkhard and Dean, 2003) are likely to be important factors that determine the risk of infection following exposure, but while relative virulence has been investigated for experimental strains (Dean et al., 1999; Pedersen et al., 2001), this information is not known for field strains of FIV causing naturally-acquired infections.

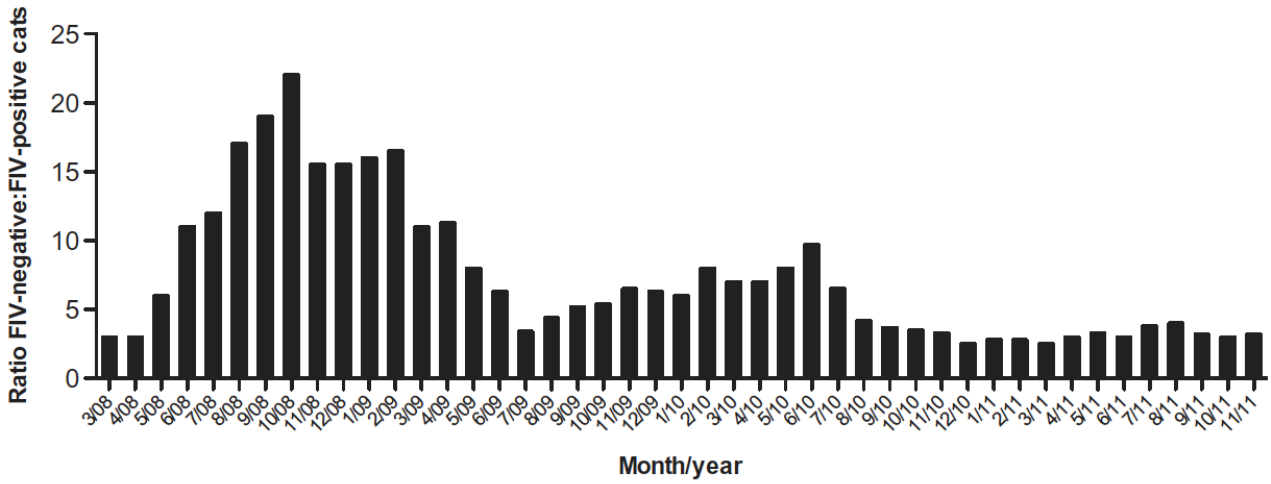

Fig. 2. Ratio of FIV-negative to FIV-positive cats cohabiting at Rescue 1 month by month (March 2008–November 2011).

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Table 3
Details of serological testing (IDEXX FIV/FeLV SNAP Combo Test) of FIV-positive queens and their weaned kittens from Rescue 2.

	Litter size and genders	Age of kittens at first test (weeks)	Test result	Age of kittens at second test (weeks)	Test result
Cat A	$n = 5$, 3 females, 2 males	9	All kittens negative	NP	NP
Cat B	$n = 3$, 2 females, 1 male	13	All kittens negative	26	All kittens negative
Cat C	$n = 4$, 3 females, 1 male	25	All kittens negative	NP	NP
Cat D	$n = 3$, 1 female, 2 males	17	All kittens negative	NP	NP
Cat E	$n = 4$, 2 females, 2 males	13	All kittens negative	NP	NP

NP, not performed.

Another important risk factor for FIV transmission in a multi-cat household is the frequency of aggression events, resulting in bite wounds (Shelton et al., 1989). All of the cats at Rescue 1 were spayed or neutered and kept indoors (except for one FIV-positive cat), thereby reducing the risk of territorial aggression; the caregiver at Rescue 1 reported no penetrating bite wounds in any of the cohabiting cats. This is supported by the results of a large prevalence study that reported sexually intact status and outdoor lifestyle as major risk factors for FIV infection (Levy et al., 2006) and other studies, which have linked cat bite wounds and abscesses with FIV-positive status (Goldkamp et al., 2008; Chang-Fung-Martel et al., 2013). Additionally, it is of note that the median age of FIV-negative cats from Rescue 1 at Test 1 was 4 months, since kittens are known to be a low risk group for FIV (Levy et al., 2006), probably because territorial aggression has not yet developed. Therefore, recommendations for 'mixed' housing of FIV-positive and FIV-negative cats should be grounded in the important considerations of feline behavior, neuter status, virology and immunology.

Because FIV is transmitted directly between cats, the ratio of cohabiting FIV-positive to FIV-negative cats is an important factor in the risk for disease transmission. Additionally, if cats are stressed, because there are large numbers kept together with few opportunities to escape from one another, the risk of fighting is likely to increase. At Rescue 1, the ratio and the total number of cats varied widely over the study period, but FIV transmission was not demonstrated in the population.

ELISA-negative, PCR-positive FIV infection was not demonstrated in the 33 cats tested using both methods at Rescue 1. Dandekar et al. (1992) reported that in some FIV-negative cats cohabiting with FIV-positive cats, FIV amplicons were detectable by PCR (Table 1). Those individuals did not develop a serological response or clinical signs of disease, although other studies have failed to substantiate this phenomenon. It is possible that delayed seroconversion could explain our results, although all but 3/29 FIV-negative cats that were tested by PCR had been cohabiting with FIV-positive cats for at least 10 months when molecular analysis was performed. Alternatively, the cats that tested FIV-negative by PCR could have had undetectable amounts of viral nucleic acid in their blood, thus making their FIV status difficult to identify using PCR.

In the present study, vertical transmission of FIV was not evident. However, since further confirmatory testing was not performed at Rescue 2, it is possible that some of the FIV-positive ELISA test results might be due to an antibody response following FIV vaccination, rather than via natural infection. However, since all these cats presented as intact strays, this was considered unlikely. This is supported by another recent study, which reported that <40% of cats had antibody titers against viral agents present in core vaccines for cats (feline panleukopenia virus, feline calicivirus and feline herpesvirus) at the time of admission to rescue shelters and factors associated with seropositivity included being neutered and owner relinquished (DiGangi et al., 2012). It is also interesting to note that vertical transmission seem to be much less common in published reports of cats with naturally-acquired FIV (Ueland and Nesse, 1992; Medeiros et al., 2012) than for experimental infections (Sellon et al., 1994; O'Niel et al., 1996; Rogers and Hoover, 1998).

This study has a number of limitations. Firstly, there were only a small proportion of cats for which longitudinal FIV serological data were available from the original 138 cats tested at Rescue 1 (Test 2 $n = 50/138$; Test 3 $n = 5/138$). This was due to a combination of an active adoption program at that shelter and the long period of time over which FIV test records were collected (since the shelter opened in October 2003 until the study was performed in November 2011). Additionally, personnel performing the tests were not blinded to previous test results, although it seems unlikely that this could influence their interpretation of the results with this type of test (Levy et al., 2004; Hartmann et al., 2007). Since all available cats were tested, knowledge of previous test results did not impact on selection of cats for testing. Finally, it is possible that misclassification error occurred if cats testing FIV-negative had been recently infected but seroconversion had not occurred by the time of FIV testing.

Conclusions

FIV serological status in cats cohabiting in a mixed household of FIV-negative and naturally infected FIV-positive cats at Rescue 1 did not change over a period of years, despite unrestricted access to one another, mutual grooming, minor episodes of aggression and sharing food and water dishes, litter pans and bedding. Serological results did not demonstrate any evidence of vertical transmission of FIV from five FIV-positive queens to their 19 weaned kittens at Rescue 2. These study findings could have implications for the recommendations made by veterinarians and shelter staff asked to advise cat owners or adopters contemplating co-housing FIV-positive and FIV-negative cats and to shelters caring for litters of kittens born to FIV-positive queens. However, careful management is required when cats are first introduced to one another, as the potential for agonistic interactions that could result in FIV transmission is increased. Because of this, it is important to determine FIV status before cats are introduced to one another and then to observe interactions until the likelihood of aggression resulting in penetrating bite wounds is considered negligible. If there is a reasonable suspicion that such agonistic interactions will occur when cats are left unsupervised, FIV-positive and FIV-negative cats should be segregated from one another.

Conflict of interest statement

The author of this paper has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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