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Abstract

Objectives Our aim was to: (i) determine the current seroprevalence of feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) in three large cohorts of cats from Australia; and (ii) investigate potential risk factors for retroviral infection.

Methods Cohort 1 (n = 2151 for FIV, n = 2241 for FeLV) consisted of cats surrendered to a shelter on the west coast of Australia (Perth, Western Australia [WA]). Cohort 2 (n = 2083 for FIV, n = 2032 for FeLV) consisted of client-owned cats with outdoor access recruited from around Australia through participating veterinary clinics. Cohort 3 (n = 169 for FIV, n = 166 for FeLV) consisted of cats presenting to Murdoch University Veterinary Hospital for a variety of reasons. Fresh whole blood was collected and tested using a commercially available point-of-care lateral flow ELISA kit that detects p27 FeLV antigen and antibodies to FIV antigens (p15 and p24) (cohorts 1 and 2), or one of two lateral flow immunochromatography kits that detect p27 antigen and antibodies to FIV antigen (p24 and/or gp40) (cohort 3). Data recorded for cats in cohort 2 included signalment, presenting complaint and postcode, allowing investigation of risk factors for FIV or FeLV infection, as well as potential geographical 'hot spots' for infection.

Results The seroprevalence of FIV was 6% (cohort 1), 15% (cohort 2) and 14% (cohort 3), while the seroprevalence of FeLV was 1%, 2% and 4% in the same respective cohorts. Risk factors for FIV infection among cats in cohort 2 included age (>3 years), sex (male), neutering status (entire males) and location (WA had a significantly higher FIV seroprevalence compared with the Australian Capital Territory, New South Wales and Victoria). Risk factors for FeLV infection among cats in cohort 2 included health status ('sick') and location (WA cats were approximately three times more likely to be FeLV-infected compared with the rest of Australia). No geographical hot spots of FIV infection were identified.

Conclusions and relevance Both FIV and FeLV remain important infections among Australian cats. WA has a higher seroprevalence of both feline retroviruses compared with the rest of Australia, which has been noted in previous studies. A lower neutering rate for client-owned male cats is likely responsible for the higher seroprevalence of FIV infection in WA cats, while the reason for the higher seroprevalence of FeLV in WA cats is currently unknown.

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Introduction

The domestic cat (*Felis silvestris catus*) is host to three known exogenous retroviruses, all with worldwide distribution: feline immunodeficiency virus (FIV; subfamily Orthoretrovirinae, genus *Lentivirus*), feline leukaemia virus (FeLV; subfamily Orthoretrovirinae, genus *Gammaretrovirus*) and feline foamy virus (FFV; subfamily Spumaretrovirinae, genus *Spumavirus*). While FFV is generally thought to be of minimal clinical significance, both FIV and FeLV result in a variety of immunological perturbations that impact on morbidity and mortality.

FeLV was first reported in 1964 following the recognition of a temporo-spatial cluster of lymphoma cases in a cattery.¹ FeLV-infected cats with persistent (progressive) infections have a 62-fold increased risk of developing lymphoma or leukaemia compared with cats not infected with FeLV, with FeLV-B infection responsible for the majority of FeLV-induced lymphoma.^{2,3} FeLV-infected cats with transient (regressive) infections, despite apparently clearing the viraemia and 'recovering', may still be at increased risk of developing lymphoma; one study found 22% (19/86) of lymphomas were PCR-positive for FeLV provirus, despite the absence of FeLV antigenaemia.^{4,5} FeLV infection can also cause severe non-regenerative macrocytic anaemia (FeLV-A) and aplastic anaemia (FeLV-C), the latter often being fatal within weeks.² The survival rate for cats persistently infected with FeLV is poor, with 90% dead within 3 years.⁶

FIV was first isolated in 1986 following the investigation of an immunodeficiency syndrome in a cat colony.⁷ Cats experimentally infected with FIV have variable immunosuppression, due, in part, to CD4+ lymphocytopenia, and are at increased risk of developing high grade B-cell lymphomas compared with cats not infected with FIV.⁸⁻¹⁰ One study of client-owned cats in Australia diagnosed with lymphoma found 50% were FIV-positive using Western blot analysis, suggesting strong evidence for FIV contributing to lymphomagenesis.¹¹ In an Australian study of 911 cats, the prevalence of FIV among 'sick' cats was approximately three times that of 'healthy' cats (21% vs 8%).¹² However, a more epidemiologically rigorous serosurvey did not demonstrate an unequivocal or immediate impact of FIV infection on feline health, instead finding equal FIV prevalence among 'sick' and 'healthy' Australian cats (8% vs 8%).¹³

Transmission of FeLV only requires close contact in the form of allogrooming or shared food and watering stations, although bite wounds are an increasingly recognised source of FeLV transmission.^{6,14,15} Vertical transmission of FeLV in the field is common and associated with high kitten mortality.⁶ Transmission of FIV is usually via the inoculation of virus-laden saliva subcutaneously as a result of bite wounds.^{13,16,17} Vertical transmission of FIV has been demonstrated experimentally,

although in the field this mechanism appears uncommon.¹⁸⁻²¹

In Australia, FIV infection is considerably more prevalent than FeLV infection in healthy client-owned cats (8% vs 0-2%).^{12,13} Similarly, New Zealand and Singapore have reported higher seroprevalences of FIV compared with FeLV (10% vs 6% in a convenience sample of sick cats in New Zealand [this difference between FIV and FeLV seroprevalences in New Zealand cats is likely to be greater than first reported; one of the authors later discovered, using PCR, that many of the FeLV-positive results were actually false-positives {personal communication}]; 16% vs 9% in healthy cats tested at a Singaporean veterinary clinic).^{22,23} The disparity between FIV and FeLV infection rates in domestic cats in Australia, New Zealand and Singapore seems out of step with other developed nations in Europe and North America, where the infection rates of FIV and FeLV are comparable (3% and 2% in North America,²⁴ 4% and 3% in Canada,²⁵ 6% and 5% in the UK,²⁶ and 3% and 4% in Germany,¹⁴ respectively).

The aim of this study was to determine the seroprevalence of FIV and FeLV in three different Australian feline cohorts: cats surrendered to a rescue facility (shelter) in Western Australia (WA) (cohort 1), client-owned cats recruited from around Australia through participating veterinary clinics (cohort 2) and cats presenting to Murdoch University Veterinary Hospital (MUVH, Perth, WA) (cohort 3) for a variety of reasons (mostly illness-related; this additional cohort was recruited to provide further insights into the high seroprevalence of FIV and FeLV in WA detected in the preliminary data analysis). Detailed information was recorded for cats in the second cohort, which permitted investigation of risk factors for retroviral infection, as well as the use of spatial statistical methods to identify potential geographic 'hot spots' of infection in Australia.

Materials and methods

Sample population

Cohort 1 consisted of cats surrendered to a shelter on the west coast of Australia in Perth, WA, between January 2011 and March 2013. Entire male cats older than 7 months of age were tested routinely, while entire female cats older than 7 months of age were tested at the discretion of the attending veterinarian (personal communication). Age was determined either by paperwork completed by the surrendering owner or estimated by the veterinarian, based on dentition.

Cohort 2 consisted of client-owned cats recruited through participating veterinary clinics in Australia between January 2012 and December 2012. Tasmania and the Northern Territory were not included in the study design. Boehringer Ingelheim technical representatives offered to supply selected clinics with up to 30

free point-of-care FIV/FeLV test kits, on the condition that veterinary staff recorded in a spreadsheet test results and basic information, including signalment, postcode, reason for presentation and a subjective assessment of 'sick' vs 'healthy' on all cats sampled. Veterinarians were instructed to test cats only if individuals were 2 years of age or older (although this criterion was not strictly adhered to), had some level of outdoor access and had not been vaccinated against FIV. This scheme was part of a Boehringer Ingelheim marketing programme to raise the profile of a FIV vaccine (Fel-O-Vax FIV; Boehringer Ingelheim) in Australia by demonstrating presence of FIV infection to clinicians in their local area, and thus to cat owners. Clinics selected included those that already recommended FIV vaccination and others that did not routinely recommend FIV vaccination owing to a perceived low prevalence of FIV in their vicinity. A free map of the local area displaying the location of FIV-positive cats was offered as an inducement to participating clinics at the conclusion of the study with the intent of encouraging owners to vaccinate against FIV (supplementary material).

Cohort 3 consisted of cats presenting to MUVH between January 2011 and December 2013. The majority were cats presenting to the emergency or feline medicine units for signs of non-specific illness; other reasons for FIV/FeLV testing included health assessments of stray animals or prior to blood donation or commencement of immunosuppressive therapy.

Serological testing

Whole blood was collected by cephalic or jugular venepuncture for immediate in-clinic testing. All cats in cohort 1 and 2 were tested using SNAP FIV/FeLV Combo (IDEXX Laboratories), according to the manufacturer's instructions. This kit is a lateral flow enzyme-linked immunosorbent assay that detects antibodies to FIV matrix protein (p15) and FIV capsid protein (p24), and FeLV antigen (specifically, core viral capsid protein p27). Cats in cohort 3 were tested using either Witness FeLV/FIV (Zoetis Animal Health) or SensPERT FeLV/FIV (VetAll Laboratories). Both of these kits use lateral flow immunochromatography to detect antibodies to FIV glycoprotein (gp40) and FeLV antigen (p27) (Witness), or antibodies to FIV capsid protein (p24) and FIV glycoprotein (gp40), and FeLV antigen (p27) (SensPERT).

Data collection

Results from cats in cohort 1 were entered into a database at the time of testing by veterinary staff. A summary of results was retrieved in May 2013 using a summary search function (Animal Shelter Manager Version 2.8.12). Pertinent data such as signalment, medical history, vaccination history and information on previous outdoor access was unavailable for these cats.

Results from cats in cohort 2 were entered into a spreadsheet at the time of testing by veterinary staff, collated at the end of the testing period by a Boehringer Ingelheim employee and then supplied to the first author for analysis. Signalment information (excluding breed), clinic postcode, primary presenting complaint and a subjective assessment of 'healthy' vs 'sick' made by the attending veterinarian were recorded alongside the cat's FIV and FeLV results. Based on the reason for presentation, cats in cohort 2 were reclassified as 'healthy' or 'sick', according to previously published definitions.^{12,13} 'Healthy' cats were those for whom the purpose of blood collection was not disease investigation; rather, it was as part of a routine health check, for routine testing prior to the dispensing of behaviour modifying medication, for routine pre-anaesthetic testing prior to sedation or general anaesthesia for neutering, grooming, dental disease or cat fight abscess treatment, or investigation and treatment of traumatic injuries. Dental disease was not graded by the attending veterinarian and so this category may have included minor teeth scaling and polishing to remove tartar, as well as extensive extractions attributable to periodontal disease. 'Sick' cats were those for whom the reason for presentation was suggestive of systemic illness, such as vomiting, diarrhoea, weight loss, respiratory signs, neoplasia and severe illness warranting euthanasia. Cats were classified as 'unknown' if the reason for presentation did not easily fit either the 'healthy' or 'sick' definitions.

Results from cats in cohort 3 were entered into the cat's medical records at the time of testing by the attending veterinarian and a summary of results retrieved in May 2015 by searching for invoiced FIV and FeLV point-of-care test kits (RxWorks Version 4.7.3200).

Statistical analysis

Numerical analyses were performed using a commercial statistical software package (GenStat, 16th edition for Windows; VSN International) with *P* values <0.05 considered significant, and 95% confidence intervals (CIs) were calculated based on a normal approximation and the Wald method (Microsoft Excel 2010 for Windows). Probability of infection was used, where possible, as the measured outcome was binomial. Univariate and multivariate logistic regression modelling was performed to determine the effect of age, sex, neutering status, health assessment ('healthy' vs 'sick') and location (state/territory) on the retroviral status of cats in cohort 2. A two-tailed Fisher's exact test was used to investigate whether entire male cats were over-represented in WA in cohort 2. The two-sample *z*-test was used to compare FIV seroprevalence between 'healthy' and 'sick' cats in cohort 2, using an online calculator (<http://epitools.ausvet.com.au/content.php?page=z-test-2>). Potential geographical hot spots of FIV infection based on postcode were

investigated using the scan statistic (SaTScan version 7). A scanning window of 5% of the study area size and a Bernoulli model (case-control) was used.

Results

Cohort 1 (shelter cats, WA)

Of 2151 cats tested, 124 were FIV-positive (6%; 95% CI 4.8–6.7). Of 2241 cats tested, 22 cats were FeLV-positive (1%; 95% CI 0.6–1.4). We were unable to determine the FIV/FeLV co-infection rate for this cohort, owing to limitations with the summary search function.

Cohort 2 (client-owned cats, Australia)

Sample population A total of 2222 cats were recruited from 130 veterinary clinics in five states and one territory of

Australia (Figure 1). Cats with incomplete details recorded were excluded from the final analyses, as were kittens 6 months of age or younger, owing to the possibility of maternal antibodies giving false-positive results with FIV testing.¹⁶ Some cats had a FIV result recorded but no FeLV result. Ultimately, 2083 cats remained available for analysis, of which 2032 also had a recorded FeLV result.

The age of cats recruited ranged from 7 months to 22 years (median age 6 years; interquartile range [IQR] 3–11 years). Castrated male cats were the most common category (974/2083; 47%), followed by spayed female cats (671/2083; 32%), entire male cats (245/2083; 12%) and entire female cats (193/2083; 9%). Overall, there was a gender bias resulting in more males (1219/2083; 59%) than females (864/2083; 41%) being tested (Figure 2).

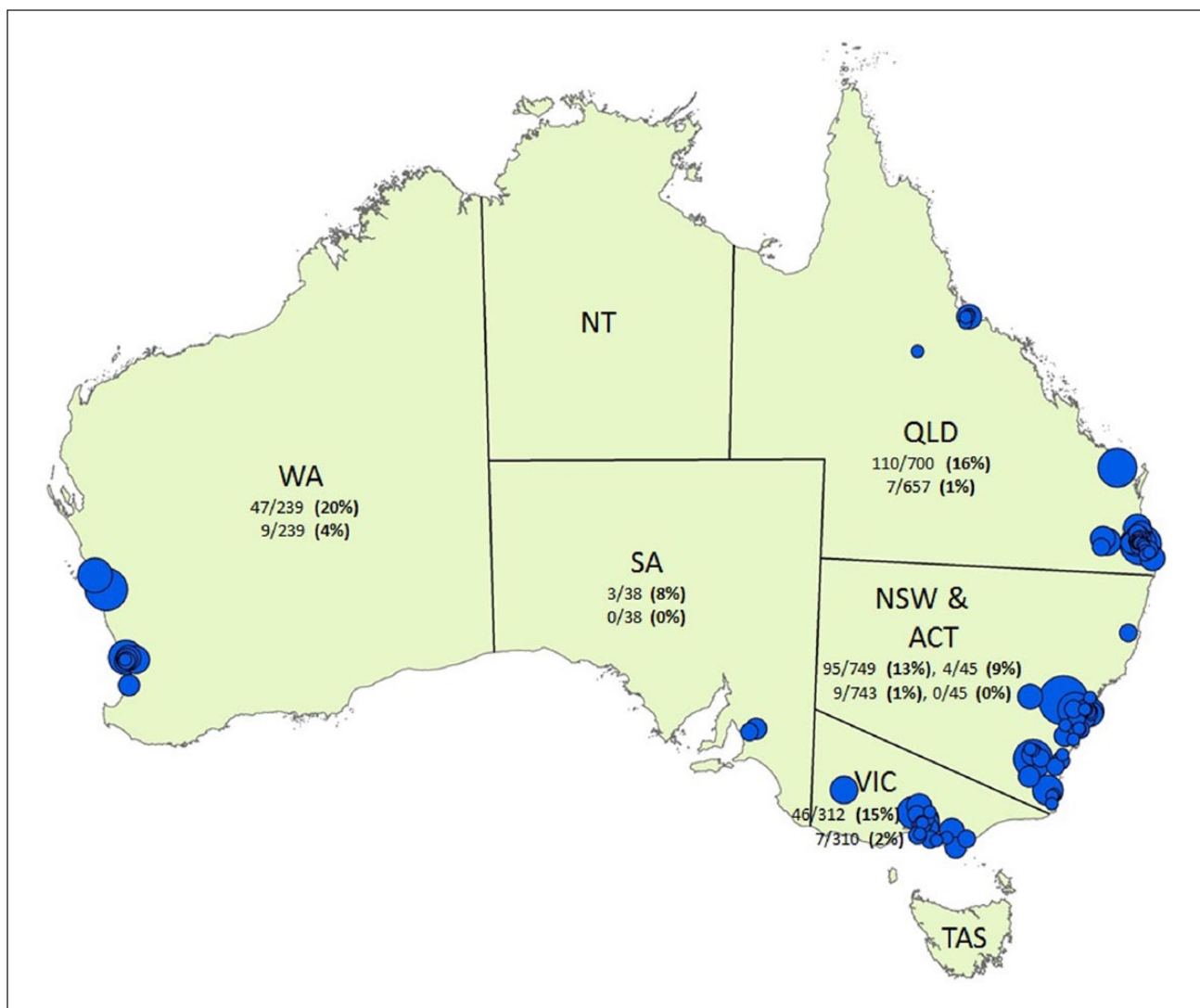


Figure 1 Map of Australia showing the distribution of client-owned cats recruited for testing (cohort 2). A larger circle indicates a higher number of cats sampled in that area. Only one poorly populated island state (TAS = Tasmania) and one sparsely populated territory (NT = Northern Territory) were not included in the study. Feline immunodeficiency virus (top line) and feline leukaemia virus (bottom line) seroprevalence rates are shown for each state/territory. ACT = Australian Capital Territory; NSW = New South Wales; VIC = Victoria; QLD = Queensland; SA = South Australia; WA = Western Australia

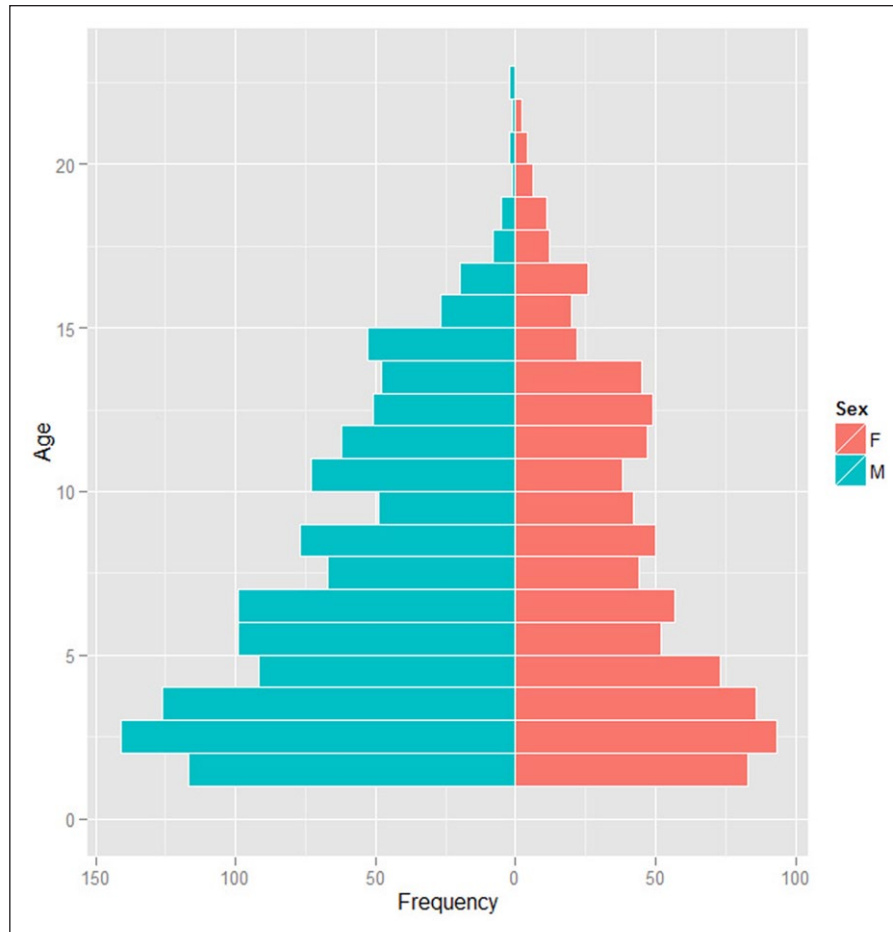


Figure 2 Age and sex pyramid for client-owned cats recruited for testing (cohort 2), highlighting the skew towards male cats. The age of cats is displayed on the y-axis, while the number of cats of each sex is displayed on either side of the x-axis. F = female; M = male

Entire male cats were significantly over-represented in WA compared with the rest of the country (54/239 [23%] vs 191/1844 [11%]; $P = 0.001$).

Serological testing Of 2083 cats tested, 305 were FIV-positive (15%; 95% CI 13.1–16.2). Of 2032 cats tested, 32 were FeLV-positive (2%; 95% CI 1.0–2.1) (Figure 1; Table 1). Of the 32 FeLV-positive cats, 11 also tested FIV-positive (34%), giving a FIV/FeLV co-infection rate of 11/2032 (0.5%; 95% CI 0.2–0.9). The median age of FIV-infected cats was 7 years (IQR 4–11 years). The median age of FeLV-infected cats was 6 years (IQR 3–10 years). FIV and FeLV seroprevalence rates by location (state/territory), sex and neutering status are provided as supplementary material.

Risk factors for FIV seropositivity The seroprevalence of FIV infection was significantly higher in cats older than 3 years of age compared with cats younger than 3 years of age ($P < 0.001$). Male cats were significantly more likely than female cats to be FIV-infected ($P < 0.001$),

while entire male cats were significantly more likely than castrated male cats to be FIV-infected ($P = 0.001$).

When FIV seroprevalence was assessed using a multivariate model to account for the significant effects of age, sex and neutering status, a significant difference between sampling locations was found ($P = 0.03$). Specifically, the Australian Capital Territory (ACT), New South Wales (NSW) and Victoria had a significantly lower FIV seroprevalence compared with WA, while ACT and NSW had a significantly lower FIV seroprevalence compared with Queensland. When WA was compared with the rest of the country, cats domiciled in that state were significantly more likely to be FIV-infected (odds ratio 1.7) (Figure 3). Although South Australia had the lowest recorded FIV seroprevalence (3/38; 8%), the low sample number and resulting large SE precluded this difference from reaching statistical significance.

The seroprevalence of FIV infection was not significantly different between 'healthy' and 'sick' cats using the aforementioned definitions (14% vs 16%; $P = 0.17$), although when cats classified with dental disease were

excluded from analysis there was a trend towards significance ($P = 0.06$). When the attending veterinarian's assessment of health status was considered, however, the prevalence of FIV infection among 'sick' cats was almost twice that of 'healthy' cats (11% vs 20%; $P < 0.0001$).

One potential geographical hot spot of FIV infection was identified in WA (postcodes 6024, 6060, 6090; $P = 0.06$). When investigated further, this cluster of infections was found to be the result of biased sampling, with a higher proportion of entire cats sampled compared with the rest of cohort 2 ($P < 0.001$). Socioeconomic data from the Australian Bureau of Statistics (ABS; www.abs.gov.au [2011 census data used]) for these postcodes was examined and compared with ABS data for other postcodes in cohort 2; no significant differences were found

Table 1 FIV and FeLV seroprevalence among client-owned cats (cohort 2) by location (raw data)

Location	FIV seroprevalence	FeLV seroprevalence
ACT	4/45 (9)	0/45 (0)
NSW	95/749 (13)	9/743 (1)
VIC	46/312 (15)	7/310 (2)
QLD	110/700 (16)	7/657 (1)
SA	3/38 (8)	0/38 (0)
WA	47/239 (20)	9/239 (4)
Total	305/2083 (15)	32/2032 (2)

Data are n (%)

ACT = Australian Capital Territory; NSW = New South Wales;

VIC = Victoria; QLD = Queensland; SA = South Australia;

WA = Western Australia; FIV = feline immunodeficiency virus;

FeLV = feline leukaemia virus

between the cluster postcodes and other postcodes for socioeconomic disadvantage ($P = 0.74$), resources ($P = 0.74$) or education ($P = 0.94$).

Risk factors for FeLV seropositivity Age, sex and neutering status were not found to be risk factors for FeLV infection ($P = 0.87$, $P = 0.50$ and $P = 0.63$, respectively), and sampling location only just failed to reach significance ($P = 0.06$). When results for WA were compared with the rest of the country, cats from that state were approximately three times as likely to be FeLV-infected (odds ratio 3.0) (Figure 3). ACT did not record any FeLV-infected cats (0/45), while WA recorded the highest seroprevalence (9/239; 4%). The seroprevalence of FeLV infection was approximately three times higher among 'sick' cats than 'healthy' cats, using both the aforementioned definitions and the attending veterinarian's assessment of health status (1% vs 3%, $P = 0.02$; 1% vs 3%, $P < 0.001$, respectively).

A summary of significant risk factors for FIV and FeLV infection of cats in cohort 2 is provided in Table 2.

Cohort 3 (cats presenting to MUVH, WA)

Sample population A total of 170 cats were tested for FIV and/or FeLV, ranging in age from 2 months to 19 years (median age 6 years; IQR 2–10 years). These cats comprised 80 castrated males (47%), 66 spayed females (39%), 17 entire males (10%) and seven entire females (4%). Most were domestic crossbred cats (127/170; 75%); the remainder comprising a range of pedigree breeds. The majority of cats were tested as part of a medical work-up for non-specific illness (114/170; 67%), followed by testing prior to commencement of

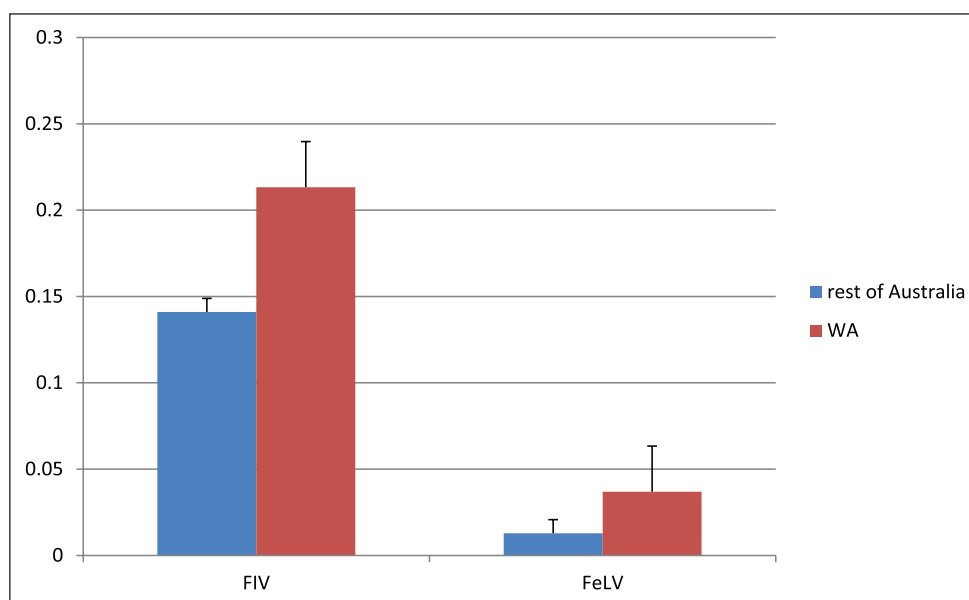


Figure 3 Feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) seroprevalence among client-owned cats (cohort 2) for Western Australia (WA) compared with the rest of Australia (model adjusted data). The y-axis shows the probability of FIV infection at a fixed point in time. SE bars are shown

Table 2 Summary of statistically significant risk factors ($P < 0.05$) for retroviral infection among client-owned cats (cohort 2)

FIV infection	FeLV infection
Age (>3 years)	Health status ('sick')
Sex (male)	Location (Western Australia)
Neutering status (entire males)	
Location (Western Australia, Queensland)	

FIV = feline immunodeficiency virus; FeLV = feline leukaemia virus

immunosuppressive therapy (31/170; 18%), testing prior to blood donation (12/170; 7%) health assessment of stray animals (11/170; 7%) and routine testing prior to FIV/FeLV vaccination (2/170; 1%).

Serological testing Of 169 cats tested for FIV, 24 were positive (14%; 95% CI 8.9–19.5). FIV-positive cats ranged from 2 months to 13 years (median age 4 years; IQR 2–8 years), comprised mostly of domestic crossbred cats (19/24; 79%) with a preponderance of male cats (14 castrated males, seven entire males, two spayed females and one entire female).

Of 166 cats tested for FeLV, seven cats were positive (4%; 95% CI 1.2–7.3). FeLV-positive cats ranged from 10 months to 8 years in age (median age 4 years; IQR 2–8 years), comprised mostly of domestic crossbred cats (6/7; 86%) and entirely male cats (four castrated males and three entire males). Of the seven FeLV-positive cats, four also tested FIV-positive (57%), giving a FIV/FeLV co-infection rate of 4/165 (2%; 95% CI 0.1–4.7 [165/170 cats had both FIV and FeLV results recorded]).

Discussion

This is the largest study to date of FIV and FeLV infection among Australian cats and provides important epidemiological information regarding feline retroviral infections in Australia. Prior to the current study there had been one reported investigation of retroviral infection in Australian shelter cats. This was an underpowered study of only 20 cats from Melbourne, Victoria, which found six cats, with a median age of 3 years, to be FIV-positive (30%).²⁷ FeLV status was not investigated in that study.²⁷ Consequently, a larger study into the retroviral status of Australian shelter cats has been long overdue. Results from feline cohort 1 in the current study confirmed the notion that FIV infection is more common in Australia (6% in the WA shelter) than North America, with a large US-based study finding the seroprevalence of FIV infection among relinquished shelter cats to be just 1%.²⁴

While there has been a paucity of studies investigating the retroviral status of Australian shelter cats, there have been several investigations into the retroviral

status of client-owned cats. These studies vary considerably in relation to location, design and recruitment, resulting in considerable variation in the reported seroprevalences of FIV and FeLV infection.^{12,13,27–33} The seroprevalence of FIV infection in these prior studies varies between 0–29% (for 'healthy' cats) and 4–32% (for 'sick' cats). The seroprevalence of FeLV infection in these prior studies varies between 0–7% (for 'healthy' cats) and 0–11% (for 'sick' cats) (Tables 3 and 4). Interestingly, like the last Australian FIV serosurvey,¹³ the current study did not find a difference in FIV seroprevalence between 'healthy' and 'sick' client-owned cats in cohort 2, using a similar classification system. This finding does not equate to FIV infection being apathogenic in Australian cats, but rather that a different study design targeting specific disease associations (eg, B-cell lymphoma), older cats (due to the long asymptomatic phase of FIV infection) and different wild strains (due to variability in pathogenicity) is required to investigate the impact of FIV infection on mortality and morbidity. When the attending veterinarian's assessment of 'healthy' or 'sick' was considered, however, a significant difference in FIV seroprevalence was found, suggesting that elements of health assessment are subjective, possibly intuitive and without doubt informative, although not easily captured by strict objective criteria. The current study found a significant difference in FeLV seroprevalence between 'healthy' and 'sick' client-owned cats in cohort 2, regardless of the classification system utilized, reflecting the well-known impact of FeLV infection on feline health.

The most novel finding from this investigation was the higher seroprevalences of both FIV and FeLV in WA cats in cohort 2 compared with the rest of Australia. Two previous, underpowered studies investigated FIV and FeLV infection in WA cats (Tables 3 and 4): a 1990 study found seroprevalence rates of 29% (FIV) and 7% (FeLV) in 'healthy' cats, and 28% (FIV) and 11% (FeLV) in 'sick' cats;³⁰ while a 1993 study found seroprevalence rates of 24% (FIV) and 6% (FeLV) in 'sick' cats.³¹ The FIV and FeLV infection rates reported in the current study for WA cats in cohort 2 (20% and 4%, respectively) appear consistent with earlier studies, despite the availability of a FIV vaccine in Australia since 2004 (Fel-O-Vax FIV; Boehringer Ingelheim). In the current study, cats in cohort 2 domiciled in WA were 1.7 times more likely to be FIV-infected, and 3.0 times more likely to be FeLV-infected compared with the rest of the country. The FIV seroprevalence disparity in cohort 2, based on location, might be attributable to the significantly higher proportion of entire male cats encountered in WA compared with elsewhere, a trend that may actually be a true reflection of pet ownership in WA rather than a sampling bias. Previous demographic studies have reported a lower neutering rate of client-owned male cats in Perth (WA) compared with Sydney (NSW) (82% vs 96%),^{34,35}

Table 3 Summary of previous Australian studies investigating FIV and progressive FeLV infection among 'healthy' cats

Reference	Location	Study design	Age	FIV prevalence	FeLV prevalence
Sabine et al (1988) ²⁸	Sydney, NSW	'Healthy' cats (n = 30), serum/plasma supplied by Webster's Vaccine Company	NP	2/30; 7%	2/30; 7%
Robertson et al (1990) ³⁰	Perth, WA	'Healthy' client-owned cats (n = 72), recruited by random selection of households from the Perth electoral rolls	NP	21/72; 29%	5/72; 7%
Malik et al (1997) ¹²	Sydney, NSW	'Healthy' client-owned cats (n = 200), prospective sampling from four veterinary clinics	Median age 4 years	15/200; 8%	4/200; 2%
Norris et al (2007) ¹³	Sydney, NSW	'Healthy' client-owned cats (n = 170), prospective sampling from 3 veterinary clinics stringently designed to reflect a typical hospital population	Median age 7 years	13/170; 8%	4/170; 2% (unpublished data)
Beatty et al (2011) ⁴¹	Sydney, NSW	'Healthy' client-owned cats (n = 169), most acquired from rescue societies), prospective sampling from 3 veterinary clinics	Mean age 3 months (all < 1 year)	0/169; 0%	0/169; 0%
Chang-Fung-Martel et al (2013) ³³	Townsville, QLD	'Healthy' cats (n = 96), door-to-door survey using a random sampling approach, saliva collected	Median age 5 years	10/96; 10%	NP

NSW = New South Wales; QLD = Queensland; WA = Western Australia; NP = not provided; FIV = feline immunodeficiency virus; FeLV = feline leukaemia virus

consistent with the current observations. In contradistinction, the FIV seroprevalence of WA cats in cohort 3 (14%) was similar to the national FIV seroprevalence of cats in cohort 2 (15%), presumably owing to a similar percentage of entire male cats being sampled in both groups (10% [cohort 3] vs 12% [cohort 2]), and as lifestyle information was not recorded, possibly the inclusion of some cats in cohort 3 without outdoor access (and therefore at reduced risk of FIV infection). It is not clear from the current data why the high proportion of entire male cats sampled in WA in cohort 2 (23%) was not observed in WA cats sampled in cohort 3 (10%). The reason for the higher FeLV seroprevalence in WA cats in both cohorts 2 and 3 compared with the rest of the country (4% and 4% vs 1%) is currently unknown and may indicate a true difference in risk of exposure to the virus. This possible increased risk of retroviral infection in WA cats should be the subject of future investigations, but, in the interim, it would be prudent for WA veterinarians to conduct a public awareness campaign to highlight the need for early neutering of male and female cats prior to sexual maturity and to vaccinate routinely young cats against FeLV.

The difference in FIV seroprevalence between shelter and client-owned cats in the current study (ie, cohort 1 vs cohort 2), and particularly the lower FIV seroprevalence in WA shelter cats compared with WA client-owned cats,

likely reflects a difference in demographics between the two cohorts. Although signalment details for shelter cats were unavailable, and Australian data on characteristics of cats entering shelters is lacking, one US study found 639/1200 (53%) adult cats (6 months of age or older) entering the shelter were younger than 3 years of age.³⁶ If this trend is also true for Australian shelters, which we think likely, and as the cumulative risk for acquiring FIV infection increases with age, it is reasonable to assume the lower seroprevalence of FIV in shelter cats compared with client-owned cats was due to a lower median sampling age in cohort 1 compared to cohort 2. Furthermore, based on previous Australian studies, it is likely that around a quarter of shelter cats sampled were previously housed exclusively indoors and therefore had very low, if any, exposure to FIV compared with the client-owned cats, which because of the study design, all had some level of outdoor access.^{33,35} Although the skew towards male cats in cohort 2 (59% vs 41%) would have resulted in a slight overestimation of FIV seroprevalence, the same was likely true for cats in cohort 1, owing to the described sampling bias towards males.

This is the first FIV seroprevalence study conducted since the introduction of the FIV vaccine in Australia in October 2004 (Norris and collaborators tested blood specimens collected prior to the release of the vaccine). FIV vaccination results in the production of FIV antibodies indistinguishable from those used for the diagnosis of

Table 4 Summary of previous Australian studies investigating FIV and progressive FeLV infection among 'sick' cats

Reference	Location	Study design	Age	FIV prevalence	FeLV prevalence
Sabine et al (1988) ²⁸	Sydney, NSW	'Sick' cats (n = 23), convenience sample using serum/plasma sent to VPDS, The University of Sydney with many specimens dating back to the 1970s	NP	1/23; 4%	2/23; 9%
Belford et al (1989) ²⁹	QLD and northern NSW	'Sick' cats and in-contact cats (n = 65), convenience sampling using serum/plasma sent to VPS from cats suspected to be FIV infected based on clinical or laboratory findings (break up of sick vs in-contact cats not specified)	NP	21/65; 32%	NP
Robertson et al (1990) ³⁰	Perth, WA	'Sick' client-owned cats (n = 211), convenience sample using serum sent to MUVH Clinical Pathology Laboratory for diagnostic work up of clinical disease (not specifically suggestive of FIV)	NP	59/211; 28%	23/211; 11%
Friend et al (1990) ²⁷	Melbourne, VIC	'Sick' cats (n = 467, consisting of 447 client-owned and 20 shelter cats), convenience sample using serum sent to CVDL or SVS, most cats displaying clinical disease compatible with immunodeficiency	NP	120/467; 26%	16/467; 3%
Thomas et al (1993) ³¹	WA	'Sick' client-owned cats (n = 326), convenience sample using blood sent to a private laboratory for diagnostic work up of clinical disease	NP	78/326; 24%	21/326; 6%
Malik et al (1997) ¹²	NSW	'Sick' client-owned cats (n = 894), convenience sample using serum sent to a private clinical pathology laboratory for diagnostic work up of suspected immunodeficiency (not all samples tested for both FIV and FeLV)	NP	148/711; 21%	11/761; 1%
Winkler et al (1999) ³²	Adelaide, SA	Client-owned cats of unknown health status (n = 389), convenience sample using serum sent to VPS (presumably cats 'sick' and sampled for diagnostic work up of their illness)	NP	39/389; 10%	NP
Norris et al (2007) ¹³	Sydney, NSW	'Sick' client-owned cats (n = 170), prospective sampling from three veterinary clinics stringently designed to reflect a typical hospital population, cats were 'systemically unwell' and sampled for diagnostic work up of their illness	Median age 7 years	14/170; 8%	4/170; 2% (unpublished data)
Beatty et al (2011) ⁴¹	Sydney, NSW	'Sick' client-owned cats (n = 75), convenience sample using cats presented to VCCC for further work up of anaemia, cytopenia, lymphoma and other illnesses	Mean age 11.5 years	8/75; 11%	0/75; 0%

NSW = New South Wales; VIC = Victoria; QLD = Queensland; SA = South Australia; WA = Western Australia; NP = not provided; MUVH = Murdoch University Veterinary Hospital; CVDL = Central Veterinary Diagnostic Laboratory; SVS = School of Veterinary Science, University of Melbourne; VCCC = Valentine Charlton Cat Centre, University of Sydney; FIV = feline immunodeficiency virus; FeLV = feline leukaemia virus

FIV infection when using SNAP FIV/FeLV Combo,^{37,38} so that in the absence of additional testing an overestimation of FIV seroprevalence is possible. However, cats surrendered to shelters have typically received a lower level of care than non-surrendered cats,³⁹ and thus are less likely to have been vaccinated against FIV. Furthermore, although not specifically stated in the study instructions provided to veterinary clinics by Boehringer Ingelheim, it is our presumption that cats vaccinated against FIV would not have been selected by veterinarians for testing. It therefore seems unlikely that previous FIV vaccination would have caused sufficient false-positive antibody test results to impact the reported FIV seroprevalence substantially for either shelter or client-owned cats.

Seroprevalence studies for FeLV in Australia are affected by the low FeLV infection rate in the general cat population and the resulting low positive predictive value of point-of-care antigen test kits, despite excellent sensitivity and specificity of the current generation of kits.⁴⁰ The European ABCD Guidelines recommend confirmatory testing for suspected cases of FeLV infection using proviral PCR, particularly in healthy cats without clinical signs of disease.⁶ If a true FeLV seroprevalence of 0.5–1.0% in Australia is postulated, then the occurrence of false-positive FeLV results with point-of-care antigen testing is similar to the prevalence of FeLV antigenaemia.^{41–43} Therefore, it is likely that the true FeLV infection rate for both cohorts of cats in the current study was actually lower than reported.

Age, sex and neutering status in males were important risk factors for FIV infection in client-owned cats (cohort 2), reinforcing findings from other studies and confirming that fighting between cats continues to be the main mechanism for FIV transmission, particularly in Australia where the climate permits outdoor access for most months of the year.^{13, 16} Conversely, age, sex and neutering status were not found to be risk factors for FeLV infection in client-owned cats. The susceptibility of cats to infection with FeLV has traditionally thought to be age dependent, with young cats more likely to be FeLV-infected (and older cats more likely to be FIV-infected).^{6,13} The current study was not consistent with this 'age effect' on retroviral status and instead found a similar median age for FIV and FeLV infection in client-owned cats (7 years and 6 years, respectively). This finding is consistent with recent research from Germany,¹⁴ suggesting a changing landscape for FeLV infection, where older cats are as likely to be FeLV-infected as younger cats. The resulting impact of FeLV infection on morbidity and mortality in older cats needs confirmation and further investigation.

Conclusions

This study reports the largest number of client-owned Australian cats recruited over nearly the whole continent to investigate the epidemiology of retroviral

infections. FIV continues to be a common infection of client-owned cats with outdoor access, making Australia an ideal location for testing the efficacy of the FIV vaccine in the field. FIV and FeLV are significantly more common in client-owned cats in Perth, WA, compared with the rest of the country. The reasons for this might be related to lower rates of neutering, and require further investigation and intervention. FeLV infection, although uncommon, should not be forgotten, even in older cats, as a potential cause of feline morbidity and mortality, including late 'downstream' effects such as lymphomagenesis. Owing to the low prevalence of FeLV antigenaemia in Australia, confirmatory testing by real-time PCR should always be pursued for any cat testing positive for FeLV antigen using point-of-care test kits. In cats infected with FeLV, co-infection with FIV is common.

Supplementary material

FIV testing scheme spreadsheet.

FIV and FeLV seroprevalence rates among client-owned cats by location, sex and neutering status (raw data).

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Conflict of interest Phillip McDonagh is Head of Regulatory Affairs (Animal Health) for Boehringer Ingelheim Australia.

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