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Prevalence of feline immunodeficiency virus infection in domesticated and feral cats in eastern Australia

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Serum samples from 340 pet cats presented to three inner city clinics in Sydney Australia, 68 feral cats from two separate colonies in Sydney, and 329 cattery-confined pedigree and domestic cats in eastern Australia, were collected over a 2-year period and tested for antibodies directed against feline immunodeficiency virus (FIV) using immunomigration (Agen FIV Rapid Immunomigration test) and enzyme-linked immunosorbent assay methods (Snap Combo feline leukaemia virus antigen/FIV antibody test kit, IDEXX Laboratories). Western blot analysis was performed on samples in which there was discrepancy between the results. Information regarding breed, age, gender, housing arrangement and health status were recorded for all pet and cattery-confined cats, while the estimated age and current physical condition were recorded for feral cats. The FIV prevalence in the two feral cat populations was 21% and 25%. The majority of FIV-positive cats were male (60–80%). The FIV prevalence in cattery-confined cats was nil. The prevalence of FIV in the pet cat sample population was 8% (27/340) with almost equal prevalence in 'healthy' (13/170) and 'systemically unwell' (14/170) cats. The age of FIV-positive pet cats ranged from 3 to 19 years; all FIV-positive cats were domestic shorthairs with outside access. The median age of FIV-positive pet cats (11 years) was significantly greater than the median age of FIV-negative pet cats (7.5 years: $P < 0.05$). The prevalence of FIV infection in male pet cats (21/172; 12%) was three times that in female pet cats (6/168; 4%; $P < 0.05$). With over 80% of this pet cat population given outside access and continued FIV infection present in the feral population, this study highlights the need to develop rapid, accurate and cost-effective diagnostic methods that are not subject to false positives created by concurrent vaccination against FIV. This is especially important in re-homing stray cats within animal shelters and monitoring the efficacy of the new vaccine, which has not been challenged against Australian strains. The absence of FIV within cattery-confined cats highlights the value in routine screening and indoor lifestyles. This study provides cogent baseline FIV prevalences in three cat subpopulations which can be used for appraising potential disease associations with FIV in Australia.

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Feline immunodeficiency virus (FIV) is an enveloped RNA virus affecting domestic and wild *Felidae* around the world. Within the family Retroviridae, it belongs to the subfamily *Lentiviridae* (*lentus* = slow), a group of viruses known to cause life-long infections with protracted incubation periods. As such, FIV is grouped with viruses such as human immunodeficiency virus, equine infectious anaemia virus, bovine immunodeficiency virus and caprine arthritis-encephalitis virus.

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FIV was first reported in 1987 when a previously healthy group of rescued cats in California exhibited a variety of signs suggestive of an immunodeficiency disorder (Pedersen et al 1987). Although this was the first recognition of FIV-associated disease, antibodies to FIV were subsequently detected in archived serum specimens collected as far back as 1975 in UK, 1972 in Australia and 1968 in USA (Gruffydd-Jones et al 1988, Sabine et al 1988, Shelton et al 1990).

The prevalence of FIV infection varies markedly in different cat populations within different countries. This variability in FIV prevalence is likely attributable to population density, reproductive status, age, gender and housing conditions. In particular, the frequency of free roaming, sexually intact owned or feral male cats is critical. While it is likely that there is a true difference in the prevalence of FIV infection in different geographic locations, such comparisons are made difficult by inconsistent criteria for sample selection. Australian surveys to date have produced a varied FIV prevalence with reported figures of 4.3–32% for variably defined 'sick' pet cats (Sabine et al 1988, Belford et al 1989, Robertson et al 1990, Friend et al 1990, Thomas et al 1993, Malik et al 1997), 6.7–29% for 'healthy' pet cats (Sabine et al 1988, Robertson et al 1990, Malik et al 1997), and 9% for feral cats (Winkler et al 1999).

Biting, usually associated with territorial aggression, has been recognised as the principal route of FIV transmission. The large quantity of FIV in the saliva of infected cats coupled with efficient inoculation by cats' sharp canine teeth bypass external host defences. FIV is present in whole blood, plasma, serum, cerebrospinal fluid and saliva of infected cats. Intravenous, subcutaneous or intraperitoneal inoculation of blood products from infected cats is recognised as a potential route for FIV transmission, highlighting the need for veterinarians to test donor cats prior to blood transfusion and use sterilised surgical and dental instruments and needles.

Pregnant queens in the acute phase of FIV infection may transmit virus to their offspring during the prenatal and postnatal periods (O'Neil et al 1995). Vertical transmission of FIV was formerly considered unlikely in chronically infected queens. Seroconversion prior to conception was said to confer passive immunity to neonates via colostrum (Callanan et al 1991, Wasmoen et al 1992) making vertical transmission unlikely. Recent research (Allison and Hoover 2003), however, found that FIV was transmitted to 22% of the offspring of chronically infected queens. In the early milk, high levels of FIV RNA was detected in cell free milk supernatant compared with plasma while relatively low proviral viral loads were detected in milk cells (macrophages, granulocytes and lymphocytes), suggesting that virus may be actively secreted by the mammary gland.

In anticipation of the possibility of widespread vaccination against FIV in Australia which will eclipse our ability to diagnose this infection via serum antibodies, this study was undertaken to determine the prevalence of FIV in owned, feral and cattery-confined cats.

Materials and methods

Defining the sample population

Pet cats

Serum samples from pet cats in Sydney presented to three inner city veterinary clinics (University Veterinary Centre, Sydney (UVCS), Paddington Cat Hospital (PCH) and Concord Animal Hospital) between November 2002 and September 2004 were tested for FIV antibodies. The study period predated the introduction of an inactivated FIV vaccine (Fel-O-Vax FIV Vaccine; Fort Dodge, Australia) into Australia by 1 month, thereby ensuring that FIV-positive results were not the result of vaccination.

To accurately reflect the FIV prevalence of the chosen hospital populations, a predetermined number of cats of a given age, breed and health status were selected for testing, according to the following criteria. The age distribution of the hospital populations was determined after reference to patient databases of two hospital populations for which such data was obtainable (UVCS and PCH). The age distribution of the hospital populations closely resembled that from a wider study of Sydney veterinary practices characterised previously (McGreevy et al 2002) and this age distribution was used for the current study (Table 1).

The number of cats of each breed to be tested was also determined after reference to the patient database of UVCS and PCH. The ratio of domestic to pedigree cats (UVCS 54:46; PCH 57:43), as well as the proportionate representation of individual breeds in both hospital populations was similar (±10%); therefore the average percentage for each breed in the combined hospital populations was calculated and used to determine the number of cats representing each breed in our sample population.

Table 1. The percentage of cats in each age group used to construct the pet cat sample population in this study based on previous studies into age distribution of cats accessing Sydney veterinary practices (McGreevy et al 2002)

Age (years)	Percentage of owned cat sample population
0–2	21.3
3–4	12.9
5–6	12.6
7–8	14.7
9–10	12.0
11–12	10.5
13–14	7.0
15–16	5.2
17–18	2.4
≥19	1.4
Total	100

Blood samples were further divided into two groups on the basis of the health status of the cat at the time of blood collection: group 1: 'systemically unwell' cats for which blood was taken to investigate their illness; group 2: 'healthy' cats, that were systemically well and the purpose of blood collection was not to investigate a current illness. Cats in this latter group included those presented for annual examinations and vaccination, as well as those for which blood was collected prior to the administration of certain drugs (eg, non-steroidal anti-inflammatory agents for acute trauma, anxiolytics, sedation prior to transport), and for routine screening tests prior to sedation or general anaesthesia for desexing, grooming, routine dental scaling or radiography post-acute trauma.

An equal number of blood samples were collected for each of these two groups. Within each breed category, there was an approximately equal distribution of cats between groups 1 and 2. There was a larger proportion of younger cats in group 2 ('healthy') and a larger proportion of older cats in group 1 ('systemically unwell'), which was consistent with the reference hospital population. In contributing veterinary practices, blood was taken from cats with renal disease on a regular basis. To avoid over-representation of cats with renal disease, the proportion of cats greater than 15 years with renal disease in the pet cat sample population was capped at 30%, the estimated prevalence of feline renal disease attending USA teaching hospitals (Lulich et al 1992).

Feral cats

A colony of feral cats living near a piggery in Menangle in south-western Sydney was the focus of a study by another research group (L. Hales) concerning the health and ecology of feral cats. As part of that project, cats were trapped and sedated for examination, venepuncture and fitting of radio collars between July and October 2003. In addition, a colony of feral cats living around the University of Sydney's Camperdown campus was the focus of a 'neuter and release' programme between September 2003 and September 2004. Blood samples taken as part of the programme were used in this study.

Cattery-confined cats

Breeders in the greater Sydney region were contacted by e-mail and phone and asked to participate in the study. They were also requested to invite other breeders to be involved. Posting an advertisement on a cat breeder's 'chat list' resulted in considerable interest from breeders in all states of Australia and, hence, some catteries tested were outside the targeted geographical region. At least two catteries of each breed were tested.

Sample collection

Whole blood (0.5–3 ml) from each cat was collected into serum separator tubes and/or ethylenediaminetetraacetic acid tubes and centrifuged at 12,000 *g* for 10 min. Serum or plasma was harvested, divided into 300-µl aliquots, transferred into 1.5-ml microcentrifuge tubes (Eppendorf AG, Germany) and stored at −20°C prior to testing.

Data collection

Information regarding breed, age, gender (including whether the cat was desexed or entire), medical history, physical findings, number of cats per household, whether the cat was housed exclusively indoors, or allowed both indoors and outdoors was obtained from medical records (pet cats) or cattery owners (cattery cats). For feral cats, the gender, estimated age and obvious injuries or abnormal physical findings were recorded for each cat.

Serological testing

Each serum or plasma sample was allowed to reach room temperature before testing for antibodies to FIV using two commercial test kits: the Agen FIV Rapid Immunomigration test

(AGEN Biomedical Ltd, Acacia Ridge, Queensland, Australia), and the Snap Combo feline leukaemia virus (FeLV) antigen/FIV antibody test kit (IDEXX Laboratories, Zetland, NSW, Australia). The Agen test is an immunomigration method that employs a synthetic gp 40 peptide from the FIV transmembrane region. The enzyme-linked immunosorbent assay (ELISA) used (Snap Combo FeLV antigen/FIV antibody test kit, IDEXX Laboratories, Zetland, NSW, Australia) detects antibodies captured using recombinant FIV antigens (p15 and p24) conjugated to an enzyme and contains positive and negative controls. The FeLV antigen results were noted to form a separate report to be published subsequently. Both methodologies were used according to the manufacturers' instructions and performed and interpreted on the same day. All samples were kept at −20°C and tested within 12 months of collection as recommended by the test kit manufacturers.

Based on theoretical considerations, it is possible that certain cats infected with FIV will be detected by one kit and not the other, although the majority of infected cats will produce antibodies enabling detection by both kits. Western blot analysis was performed twice on samples in which there was a discrepancy between the results of the commercial kits. The Glasgow-8 strain of FIV was grown in FL4 cells and maintained according to the protocol described by Hosie and Jarrett (Hosie and Oswald 1990). Cells were harvested at 48 h and stored at −80°C for cell lysate antigen preparation according to the method described previously (Hosie and Oswald 1990) using diluted extracts as antigen for the Western blots. Diluted cell lysates were solubilised by boiling for 3 min in buffer containing 50 mM Tris−HCl (pH 6.8), 100 mM dithiothreitol and 2% sodium dodecylsulphate (SDS). Polypeptides were separated on 10% w/v SDS-polyacrylamide gels, transferred on to nitrocellulose by a semidry method (Kyhse-Andersen 1984) and exposed first to primary antibody for 16 h and then to goat anti-cat horseradish peroxidase conjugated secondary antibody (anti-cat IgG, heavy and light chain specific antibody; Jackson Immunoresearch Laboratories, West Grove, USA) for 6 h, with appropriate washing steps. The chromagen used was 4-chloro-1-naphthol. Cats were interpreted as FIV-positive on Western blot analysis according to reactivity of antibodies with individual viral proteins according to established criteria. Sera were regarded as positive if they reacted with gp120 alone, or to three or four of the core proteins (p55, p24, p17 or p10) (Hosie and Oswald 1990). Antibodies to the transmembrane protein gp40 were not visible using this extraction technique. Each blot included a positive and a negative serum control.

In this study, a cat was classed as FIV positive if it tested positive to both the immunomigration and ELISA methods or if tested positive to either the immunomigration or ELISA methods and to the Western blot analysis. We considered the Western blot result to be definitive in determining FIV-status.

Comparison with previous FIV prevalence studies in Australia

The databases CAB-abstracts and MED-line were accessed electronically and using the keywords 'FIV' or 'feline immunodeficiency virus' all articles were examined to find all FIV prevalence studies conducted in Australia. The study design and results were examined for each Australian FIV prevalence study.

Data analysis

All analyses were performed using a commercial statistical software package (Minitab v.13.32 for Windows), with the exception of Fisher's exact test, which was available online (http://www.matforsk.no/ola/fisher.htm). For all tests, P values ≤ 0.05 were considered significant. Fisher's exact tests were used to compare the FIV prevalence between male and female cats, as well as the FIV prevalence between the health status groups 1 and 2. The median age of FIV-positive and FIV-negative cats was compared using Mann−Whitney U tests.

Results

Pet cat population

Serum samples were obtained from 340 pet cats comprising 168 females (49%) and 172 males (51%). All pet cats surveyed were desexed. Breeds included Abyssinian, Australian Mist, Birman, Bombay, British Shorthair, Burmese, Chinchilla, Cornish Rex, Dexon Rex, domestic longhair (DLH), domestic mediumhair (DMH), domestic shorthair (DSH), Himalayan, Manx, Oriental, Persian, Ragdoll, Russian Blue, Siamese, Sphinx and Tonkinese. Domestics (DSH, DMH and DLH) represented 55% (187/340) of the sample population, reflecting a similar demographic to that of the two major hospitals in this study.

The ages recorded for the cats ranged from 3 months to 21 years (median 7 years; interquartile range (IQR) 5–12 years).

The 170 cats categorised as group 1 were presented by their owner for veterinary attention for one or more of the following reasons: weight loss, vomiting, diarrhoea, inappetence, pyrexia, behavioural changes, polyuria/polydipsia, stranguria, haematuria, urethral obstruction, haematochezia, shifting lameness, hyperaesthesia, icterus, anaemia, leukopenia, mesenteric lymphadenopathy, allergic airway disease, upper respiratory infection, bacterial urinary tract infection, inflammatory bowel disease, advanced periodontal disease, stomatitis, cryptococcosis, cardiac disease, hyperthyroidism, chronic renal disease, lymphadenomegaly, oral neoplasia, nasal neoplasia, cat fight abscess, diabetes mellitus, lung adenocarcinoma, seizuring, feline infectious peritonitis, lymphocytic cholangitis, megacolon, pancreatitis, septic arthritis, allergic skin disease, recurrent conjunctivitis and keratitis. The median age of cats categorised as group 1 ('systemically unwell') was 10 years, while the median age of cats in group 2 ('healthy') was 6 years.

The housing arrangement for 61% (206/340) of cats was available from the clinical records. From these 206 cats, 82% of cats (169/206) had outside access while 18% of cats (37/206) were kept exclusively indoors. Of the 37 cats kept exclusively inside 22 (59%) were domestic cats while 15 (41%) were pedigree cats. Of the 169 cats with outside access 64 (38%) were pedigree cats and 105 (62%) were domestic cats. All FIV-positive cats in this study were DSH with access to outdoors. Therefore, 16% (27/169) of cats with known access outside were FIV positive.

The overall prevalence of FIV infection in pet cats was 8% (27/340) (Table 2). Fourteen FIV-positive cats were from group 1 ('systemically unwell'; 14/170; 8.2%) while 13 were from group 2 ('healthy'; 13/170; 7.7%). FIV-positive cats from group 1 had one or more of the following clinical signs: vomiting, diarrhoea, weight loss, anaemia, nasal discharge, haematuria, thickened gastrointestinal tract, cat fight wound, mesenteric lymphadenomegaly, leukopenia, ultraviolet induced squamous cell carcinoma of the upper lip and face, hyperthyroidism, or chronic renal disease. The age of FIV-positive cats ranged from 3 to 19 years (median 11 years; IQR 7–12.5 years). The age of FIV-negative cats ranged from 3 months to 21 years (median 7.5; IQR 3–12 years). FIV-positive cats were significantly older than FIV-negative cats ($P < 0.05$). The prevalence of FIV infection in male cats (21/172; 12%) was significantly higher than that in female cats (6/168; 4%; $P < 0.05$).

Table 2. Summary of the FIV prevalence in within subpopulations of the current study

Cat subpopulation	FIV prevalence
Pet Cats	
'Healthy'	7.7% (13/170)
'Systemically unwell'	8.2% (14/170)
Males	12% (21/172)
Females	4% (6/618)
Cats with access to outside	16% (27/169)
Cats exclusively inside	0% (0/37)
Feral cats	
Periurban	21% (10/48)
Inner city	25% (5/20)
Cattery confined cats	0%

There was a discrepancy between the immunomigration and ELISA methods in two samples only. One serum sample found to be positive for FIV using the immunomigration method and negative using ELISA was positive using Western blot analysis. Likewise, one serum sample positive for FIV using the ELISA but negative using immunomigration was positive using Western analysis. In both cases, Western analysis demonstrated antibodies against envelope protein gp120 and core protein 55, while antibodies against core proteins p24, p17 and p10 were not detected.

Feral cats

Serum samples were obtained from 48 feral cats living near a piggery in Menangle in outer south-western Sydney (75 km from Sydney's central business district (CBD)). Their estimated ages ranged from 8 weeks to 5 years. Equal numbers of male and female cats were tested. All were believed to be sexually entire DSH. The prevalence of FIV infection in this cohort was 21% (10/48) comprising six toms and four queens. All FIV-positive cats were mature adults.

Blood samples were also collected from 20 feral cats living around the University of Sydney Camperdown campus, an inner city area 4 km from Sydney's CBD. Their estimated ages ranged from 10 weeks to 4 years. All were believed to be sexually entire DSH and there were equal numbers of males and females. The prevalence of FIV infection in this cohort was 25% (5/20),

comprising four toms and one queen. All FIV-positive cats were mature adults.

There was 100% agreement between the ELISA and immunomigration methods for both cohorts of feral cats. The difference in FIV prevalence between the two feral cat populations was not statistically different.

Cattery-confined cats

Serum samples from 329 cattery-confined cats were tested for FIV antibodies. Breeds of cats tested included Australian Mist, Bengal, Birman, British Shorthair, Burmese, Burmilla, Cornish Rex, Devon Rex, DSH, Exotic, Maine Coon, Oriental, Persian, Ragdoll, Scottish Fold, Scottish Shorthair, Siamese, and Tonkinese. Of the cats tested, 110 were male and 229 were female, ranging in age from 3 months to 20 years (median 2 years; IQR 11 months to 4 years). No FIV-positive cats were detected in this cohort and there was 100% agreement between the ELISA and immunomigration methods.

Comparison with previous FIV prevalence studies in Australia

Prior to the current study there have been seven FIV prevalence studies in Australia, with results ranging from 9% in apparently healthy feral cats (Winkler et al 1999), 6.7–29% in 'healthy' pet cats (Sabine et al 1988, Robertson et al 1990, Malik et al 1997) and 4.3–32% in 'sick' cats (Sabine et al 1988, Belford et al 1989, Friend et al 1990, Robertson et al 1990, Thomas et al 1993, Malik et al 1997, Winkler et al 1999). The FIV prevalence and study design used in these Australian studies are summarised in Tables 3 and 4, respectively.

Discussion

This FIV prevalence study was stringently conducted to obtain a sampled pet cat population that reflected the target hospital population. It provides a cogent FIV prevalence of approximately 8% in both 'systemically unwell' and 'healthy' pet cats in this population which can be used for investigating potential disease associations with FIV. The current study is the first to examine FIV prevalence in cattery-confined cats. The zero FIV prevalence recorded is likely to reflect FIV screening performed by breeders when sourcing new cats and the indoor or enclosed facilities of most catteries, illustrating the effectiveness of these measures in preventing the spread of FIV. The FIV prevalence in the two distinct feral cat populations was approximately three times that of the pet populations. This highlights the threat the feral cat population poses to the >80% pet cats with outside access.

Winkler et al (1999) collected serum samples from feral cats shot in the Flinders Range National Park (South Australia) as part of an eradication campaign and determined the FIV prevalence to be 9%. The prevalence of FIV in the feral population in that study differs considerably from the current study of 21–25%. Biting is considered the major mode of FIV transmission and, therefore, a likely explanation is the difference in population density between feral

Table 3. Summary of the location and FIV prevalence in previous Australian studies

Reference	Australian city/state	FIV prevalence as detected by circulating antibodies from serum or plasma
Sabine et al (1988)	Sydney, NSW	6.7% (2/30) 'normal' pet cats, 4.3% (1/23) 'sick'pet cats, 21% (3/14) pet cats with lymphosarcoma, 20% (9/44) cats experimentally infected with FeLV
Belford et al (1989)	Queensland and NSW	32% (21/65) 'sick' pet cats with signs suggestive of FIV or cats 'in contact' with FIV positive cats
Robertson et al (1990)	Perth, Western Australia	29% (21/72) 'healthy' pet cats, 28% (59/211) 'sick' pet cats
Friend et al (1990)	Melbourne, Victoria	26% (93/357) pet cats with suspected immune deficiency, 25% (27/110) stray cats sampled as part of another study
Thomas et al (1993)	Western Australia	24% (78/326) 'sick' cats
Malik et al (1997)	Sydney, NSW	7% (14/200) 'healthy' pet cats, 21% (148/711) pet cats with suspected immune dysfunction
Winkler et al (1999)	Adelaide, South Australia	10% (39/389) domesticated cats of unknown health status, 9% (6/66) feral cats

Table 4. Summary of the study designs used in FIV prevalence studies in Australia

Reference	Details of study design
Sabine et al (1988)	Cross-sectional study using convenience based sampling of archived feline serum dating back to 1972, consisting of 'normal' cats (supplied by Webster's vaccine company), 'sick' cats (collected from Veterinary Pathology Diagnostic Services, The University of Sydney), cats from the 1970s with naturally occurring lymphosarcoma and cats experimentally infected with FeLV in a previous study.
Belford et al (1989)	Cross-sectional study using convenience based sampling of cats with clinical or laboratory findings were suggestive of FIV infection and from cats in contact with known FIV-positive cats.
Robertson et al (1990)	Cross-sectional study of randomly selected healthy cats from households on the electoral roll in which the owner was prepared for their cat to be examined by a veterinarian and tested for FIV antibodies. In addition, cross-sectional study using convenience based sampling consisting of sick cats showing any evidence of clinical disease (not specifically suggestive of FIV) and for which blood had been submitted to Murdoch University Clinical Pathology Laboratory for diagnostic purposes.
Friend et al (1990)	Cross-sectional study using convenience based sampling of 467 feline serum samples from three sources: 357 samples from cats with suspected FeLV or immune deficiency submitted to the Central Veterinary Diagnostic Laboratory (CVDL; 334 samples) and the Clinical Pathology Laboratory at the University of Melbourne (CPLUM; 23 samples) and 110 samples collected in 1980 as part of a previous study on *Chlamydophila psittaci* from cats in an animal shelter, CVDL and CPLUM.
Thomas et al (1993)	Cross sectional study using convenience based sampling of 'ill' cats from blood submitted to a private laboratory. Samples were submitted by 150 veterinarians throughout Western Australia.
Malik et al (1997)	Cross-sectional study using convenience based sampling of 200 healthy cats >1 year of age presented to veterinary clinics in the area around Sydney, NSW, for routine procedures (including cat fight injuries or abscesses, vehicular trauma, neutering, dental scaling, vaccination, grooming or boarding) and 711 submissions to a private clinical pathology laboratory, from sick cats suspected of having immune dysfunction.
Winkler et al (1999)	Cross-sectional study using convenience based sampling of 389 stored serum samples from domestic cats of unknown health status, submitted to the Veterinary Pathology Services in Adelaide, South Australia as well as 66 serum samples from feral cats shot in the Flinders Range National Park, South Australia as part of an eradication campaign.

cats in a remote national park vs those within a more densely populated region such as Sydney. While the FIV prevalence in the two feral cat populations cannot be extrapolated to represent the overall feral population in Sydney, the 70 km distance and difference in environment (inner city vs semi-rural) between populations did not produce a significant difference in results.

Previous studies in Australia have produced considerable variation in FIV prevalence between and within 'healthy' pet cats and 'sick' pet cats (Table 3). This is most likely attributable to the limitations of and variations in the study designs, the use of convenience samples, the criteria used to define 'sick' vs 'healthy' cats, as well as differences in geographical location, population density and cat husbandry practices (housing, desexing). The current study of the pet cat population, carefully designed to reflect feline hospital demographics of inner city veterinary clinics, resulted in a FIV prevalence of 7.7% in the sampled 'healthy' cat population. This study design distinguishes the present work from most previously published surveys of FIV prevalence, which were either less selective in composing their sample population, or sampled a 'convenience' population without first establishing selection criteria. Despite this distinction, our results for 'healthy' pet cats were remarkably consistent with the 7% FIV prevalence found 10 years earlier in the convenience based cross-sectional study (Malik et al 1997), the only other sizable study conducted in Sydney. Both studies have collected samples from pet cats accessing veterinary facilities and so it is uncertain how closely this reflects the general pet cat population and extrapolation of results beyond this is not possible.

The FIV prevalence found in this study of 'systemically unwell' pet cats in Sydney (8.2%) differs considerably from the 21% found previously in Sydney (Malik et al 1997). Malik and colleagues defined 'sick' cats as those with suspected immune dysfunction, while the current study defined sick or 'systemically unwell' cats as those in which blood was taken for diagnostic investigation of a clinical problem. The 170 cats categorised in group 1 ('systemically unwell') had a wide variety of presenting complaints and confirmed diagnoses, many of which are not traditionally associated with FIV infection. The current study showed an almost equal FIV prevalence in both 'healthy' and 'systemically unwell' sample populations of pet cats. This can be explained by the broader definition used to classify systemically unwell cats coupled with the frequently long period for which this virus remains preclinical or subtle in its disease manifestations. This finding does not dispute the contribution of FIV to morbidity and mortality in Australian cats (Robertson et al 1990, Gabor et al 2001). This study showed a substantially higher prevalence of FIV in males than in females and higher median age of FIV-positive cats compared with FIV-negative cats, findings that have been mirrored in studies worldwide.

Investigating the direct or indirect causative role for FIV in the pathogenesis of disease in cats has been the focus of several studies in Australia (Gabor et al 2001, Malik et al 2002, O'Brien et al 2004). The inferences made in these investigations must be re-interpreted in light of the data from the present study as comparisons were formerly made with a FIV prevalence of 21% in 'sick' cats (Malik et al 1997). Gabor et al (2001) found that 50% of cats with naturally occurring lymphosarcoma in Australia were positive for FIV antibodies, over six times the prevalence of FIV in 'healthy' and 'sick' cats as defined in the current study. On the other hand, O'Brien et al (2004) in a 20-year retrospective study of cryptococcosis in Australia, found that 21% of the 96 cats tested were FIV positive, 2.5 times the prevalence of FIV in our reference population. In light of the new data, the potential association between cryptococcosis and FIV in certain cats should be re-appraised.

There was excellent correlation between the current versions of the two commercial test kits used in this study: the Agen FIV Rapid Immunomigration test and the Snap Combo FeLV antigen/FIV antibody test kit despite differences in the antibody detected by each kit. One false-negative result with the Snap Combo was explained by the Western blot, as antibodies to p24 were not detected using this methodology in this cat. Antibodies to the envelope protein p40 are not detected using the extraction technique used to isolate antigen. When comparing different test kits and even Western blot analyses, it should be remembered that the production of antibodies to the different antigens is not consistent throughout the stages of infection.

The introduction of the new FIV vaccine into Australia in October 2004 has placed limitations on the use of antibody-based tests for the diagnosis of FIV due to the development of antibodies following vaccination that are identical to natural infection. With over 80% of this pet cat population

given outside access and continued FIV infection present in the feral population, this study highlights the need to develop rapid, accurate and cost effective diagnostic methods that are not subject to false positives created by concurrent vaccination against FIV. This is especially important in re-homing stray cats within animal shelters and monitoring the efficacy of the new vaccine, which has not been challenged against Australian strains. The absence of FIV within cattery-confined cats highlights the value of routine screening and an indoor lifestyle.

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