Seroprevalence study of Feline Coronavirus in owned and feral cats in Sydney, Australia

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Objectives i) To establish the seroprevalence of Feline Coronavirus (FCoV) infection in two defined groups of cats in Sydney: owned and feral cats; ii) to identify factors associated with an increased risk of infection with FCoV; and iii) to establish the seroprevalence and FCoV antibody titres of owned cats with immunohistochemically confirmed feline infectious peritonitis (FIP).

Design Prospective multi-institutional cross sectional study.

Procedure Serum samples from owned cats presented to three inner city veterinary clinics in Sydney and feral cats from a colony in South Western Sydney over an 11-month period were tested for FCoV antibodies using the Immunocomb® test kit. The relationship between serological score and six major factors (breed, age, gender, number of cats per household, living environment and health status) in the owned cat sample population was analysed and compared to cats with FIP.

Results The seroprevalence of FCoV infection in the sample population of owned and feral cats was 34% and 0%, respectively. The median Immunocomb® scores of DSH, Persian, Siamese and Devon Rex cats were significantly lower than that of Burmese, BSH, Abyssinian, Birman, Ragdoll and Russian Blue. The median Immunocomb® score of pedigree cats less than 2 years-of-age was significantly higher than for pedigree cats greater than 2 years-of-age. This distinction was not evident in DSH cats in these age groups. The number of cats per household at the time of blood collection had a strong positive association with Immunocomb® score. The median Immunocomb® score of cats with immunohistochemically confirmed FIP was significantly higher than cats in the sample population of owned cats but there was sufficient overlap between these two groups to make definitive diagnosis of FIP by serology impossible.

Conclusion This represents the first seroprevalence study of FCoV in Australia. The major determinants of antibody score of owned cats identified in this study were breed, age and the number of cats per household. The significant relationship between the breed of the cat and the FCoV antibody titre further supports the notion, proposed previously by the authors, that breed related differences exist in the immunological response to FCoV infection.

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Historically, there is substantial evidence that serological studies of FCoV infection provide important epidemiological insights. The first serological study of FCoV by Pedersen in 1976[1] identified a significantly higher rate of seroconversion to what was thought at the time to be FIPV, than the incidence of FIP within a population of cats in northern California. Subsequent studies by other researchers report similar findings.[2,3] It was proposed that FIP is an uncommon outcome of infection with a ubiquitous virus that causes only mild disease in the majority of cats. However, this theory appears to contradict results from experimental FIPV inoculation studies, which clearly demonstrate that the majority of cats infected developed rapidly fatal FIP. The apparent discrepancy in viral pathogenesis between natural and experimental infections prompted further investigation of the virus responsible for seroconversion of the majority of naturally infected cats that do not succumb to FIP. The eventual outcome of these efforts was the hypothesis that FIP occurred as the result of spontaneous mutation of FCoV during the course of infection – a theory that revolutionised our understanding of FIP pathogenesis.[4] The potential of FCoV serological studies to provide valuable epidemiological information is well recognised and reflected in the considerable number of serosurveys of feline populations which have been, and are continuing to be, conducted in many countries around the world.

While FCoV serology is unable to predict which cats will go on to develop FIP, it is generally accepted that the magnitude of the FCoV antibody titre is a reflection of recent viral load.[5-7] Thus, factors associated with seropositivity may be considered, indirectly, to be risk factors for FIP. Comparing the prevalence and magnitude of FCoV antibody titres within and between various feline populations may identify such factors. As well as contributing to the understanding of the epidemiology of FCoV infection and disease, it is potentially important for the prevention and control of FIP, as at-risk groups of cats can be managed to minimise FCoV loads, at least in theory.

Knowledge of the epidemiology of FIP is continually evolving. For many years, FIP has been considered a disease primarily of purebred cats living together in close confinement, such as catteries and multicat households. Intensive management of indoor cats in multicat households and catteries facilitates faecal-oral transmission of FCoV, due to increased contact with the faeces of other cats.[2,8] It has been assumed that cats with a more extensive lifestyle, for example feral and free-ranging indoor/outdoor cats that have a larger territory and bury their faeces outside, have a lower FCoV seroprevalence than indoor cats. However, two recent studies have challenged this fundamental hypothesis. Muirden[9] examined the seroprevalence of stray cats presented to the Birmingham RSPCA and found that feral or semi-feral cats are almost twice as likely to be seropositive

BSH	British Shorthair cats
CAH	Concord Animal Hospital
DSH	Domestic Shorthair cats
FCoV	Feline Coronaviruses
FeCV	Feline enteric coronavirus
FIP	Feline infectious peritonitis
FIPV	Feline infectious peritonitis virus
IFAT	Immunofluorescent antibody test
ORL	Ordinal logistic regression
PCH	Paddington Cat Hospital, Sydney
RSPCA	Royal Society for Prevention of Cruelty to Animals
UK	United Kingdom
UVCS	University Veterinary Centre Sydney

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as tame cats. Similarly, Cave and colleagues[10] studied the prevalence of FCoV antibodies in cats relinquished to a rescue facility in the UK, and found that free-ranging cats are more likely to be seropositive than indoor cats. Results from serological studies such as these continue to modify our state of knowledge and advance the understanding of the epidemiology of FCoV infections in different circumstances.

If serology is to be used as an adjunct to the diagnosis of FIP, the interpretation of serological results requires the establishment of reference ranges for different cat populations. As for all diagnostic tests, it is necessary to have some concept of 'normal' results in order to recognise and correctly interpret potentially abnormal findings.[11] It is therefore imperative that, wherever FCoV serological tests are available and marketed as aids for the diagnosis of FIP, the reference population has been characterised serologically and estimates of seroprevalence in different subsets of this population are available.

There have been no published FCoV seroprevalence studies of Australian cats to date, with the exception of a group of 69 cats tested in 1979 as part of a worldwide study.[3] Due to a paucity of information concerning the signalment, history and health status of cats tested in this study, the significance of results or relationship to the true seroprevalence in Australian cats is difficult to assess. Findings from seroprevalence studies conducted overseas are not directly transferable to Australia, as the geographic variation in estimates of FCoV seroprevalence overseas has been marked, ranging from 9% to 84%.[3,11] In addition to this, the prevalence of other viral infections in Australian cats is significantly different from cats in the UK and North America, the two areas from which the majority of FCoV seroprevalence data has been derived. These differences in seroprevalence may be indicative of the presence of strains of different virulence and infectivity in Australia (perhaps facilitated by our geographical isolation), and/or inherent differences in the Australian cat population attributable to variations in genetic constitution, husbandry and management of cats. Similar factors are likely to be also applicable when considering the seroprevalence of FCoV infections in Australia, as (i) the high rate of mutation results in many different strains of FCoV that may vary in virulence;[12,13] (ii) there is an important genetic component to FIP susceptibility;[14] and (iii) transmission of FCoV is heavily influenced by the environment and management.[6,15,16] These observations illustrate that we cannot simply assume that overseas estimates of seroprevalence are comparable to our own and there is therefore a need to investigate FCoV infections in the unique Australian environment. Indeed the results of a preliminary retrospective study by the authors showed that breed was an important additional consideration in FCoV seroprevalence in Australian cats.[17]

The aims of this study were: i) To establish the seroprevalence of FCoV infection in two defined groups of cats in Sydney: owned and feral cats; ii) to identify factors associated with an increased risk of infection with FCoV; and iii) to establish the seroprevalence and FCoV antibody titres of owned cats with immunohistochemically confirmed FIP.

Materials and methods

Defining the sample populations

Owned cats

Serum samples from client-owned cats presented to UVCS, PCH and CAH between November 2002 and September 2003 were tested for FCoV antibodies. To reflect the seroprevalence of the chosen hospital population more accurately, a predetermined total number of cats (based on estimated prevalence of 25%[1,2,4,9,10], allowable error of 5% and confidence level of 95%[18]) of each age, breed and health status (using proportional allocation) were selected for testing according to the criteria below.

The age distribution of the hospital populations was determined after reference to the patient database of the two major hospital populations from which the blood samples were collected (UVCS and PCH). This closely resembled those from a wider study of Sydney veterinary practices characterised by McGreevy and colleagues.[19] Table 1 outlines the age distribution reported by McGreevy and colleagues used in this study.

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 pure breeds, as well as the proportion of the majority of individual breeds in both of these hospital populations was similar. Therefore, the mean percentage of each breed in the combined hospital populations was calculated and used to determine the number of cats representing each breed in our sample population.

Blood samples were further divided into two groups on the basis of the health status of the cat at the time of blood collection: i) Group one: 'sick' cats for which blood was taken for haematological and/or biochemical analyses (that is for diagnostic purposes) and ii) Group two: 'healthy' cats, for which the purpose of blood collection was other than diagnostic testing. Cats in this latter group included those presented for vaccination as well as those for which blood was collected prior to the administration of certain drugs, and for screening tests prior to general anaesthesia for desexing, dental prophylaxis or radiography following acute trauma.

An equal number of blood samples was collected from each of these two groups. Within each breed tested there was an approximately equal distribution of cats between groups 1 and 2. An equal number of cats from these two groups in each age group was not actively sought, because the selection of cats based on the preceding criteria alone resulted in a larger proportion of 'healthy' cats in the younger age groups and a larger proportion of 'sick'

Table 1. The percentage of cats in each age group used to construct the owned cat sample population of this Feline Coronavirus seroprevalence study

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

cats in the older age groups, reflective of the reference population.

In contributing veterinary practices, blood was commonly taken from cats with renal disease on a regular basis for assessment of renal function. Therefore, to avoid over-representation of cats with renal disease in this project, the proportion of cats greater than 15 years-of-age with renal disease in the pet cat sample population was fixed at 30%, which is the estimated prevalence of feline renal disease in the USA.[20]

Feral cats

A 'colony' of feral cats living on a commercial pig property in south-western Sydney was the focus of a study by other researchers concerning the health and ecology of feral cats in NSW. As part of this research project, cats were trapped and sedated for physical examination, blood collection and fitting of radio collars before being released.

FIP cases

Veterinarians in Australia who had been invited to submit tissue samples for immunohistochemical analysis as part of a concurrent research project from cats strongly suspected of having FIP were encouraged also to submit blood samples from these cats for serological testing. Results from cats with immunohistochemically confirmed FIP constituted a discrete group for comparison with the client-owned cohort.

Blood sample collection

Whole blood (1 to 3 mL) from each cat presented to PCH and CAH was collected into serum separator tubes and/or EDTA tubes and centrifuged at 12000g for 10 minutes. Serum was harvested, divided into 300mL aliquots, transferred into 1.5mL microcentrifuge tubes (Eppendorf AG, Germany) and stored at –20°C prior to testing. At UVCS, excess plasma remaining after biochemical analysis of blood samples (originally collected into lithium heparin tubes) was also collected and stored, as above.

Data collection

Client owned cats

The clinical record of each patient was accessed and the following details recorded (if available): breed, age, gender, medical history, clinical signs (if any), number of cats in the household, whether the cat was housed exclusively indoors, or whether it was allowed both indoors and outdoors.

Feral cats

The gender, estimated age and any obvious injuries or abnormal physical findings were recorded for each cat. Serum obtained as part of the other research project was also used for FCoV serological testing.

Serological testing

The Immunocomb® FCoV antibody test kit (Biogal-Galed Laboratories, Israel), a commercially available in-house serological test, is based on the enzyme-linked immunosorbent assay (ELISA) technique. This test provides a semi-quantitative measure of the FCoV antibody titre present in whole blood, plasma, serum, effusion or CSF specimens. The interpretation of test results differed from the manufacturer's recommendations and was based upon the results of a previous study (Table 2).[21]

Immunocomb® kits were used according to manufacturer's instructions. Developed tests were left to air dry for a minimum of 3 hours before being individually scanned (Canonscan 650U, Canon, Tokyo). A software program developed by the manufacturer was used to measure the amount of light absorbed by each of the three spots, on a scale from 0 to 255, on each scanned test strip. The shade of colour of the test spot (proportional to the concentration of antibodies directed against FCoV present in the sample) was then converted into three values – net absorbance, relative absorbance (comparing the absorbance of the test spot with the absorbance of the positive reference spot), and a clinical score. The clinical score, which ranged from 0 to 6, was used in our analysis. Clinical scores of 0, 1 and 2 were considered negative, while scores greater than 2 were considered positive. Cats with FCoV antibody scores greater than 2 are likely to be shedding FCoV at that time.

Table 2. Modified interpretations to Immunocomb® Feline Coronavirus test kit clinical scores used in this study, according to Addie.[20,21]

Clinical score	Manufacturer's interpretation	Modified interpretation
0	Negative	Negative
1	Low positive	Negative
2	Low positive	Negative
3	Low positive	Low positive
4	Medium positive	Low positive
5	Medium positive	Medium positive
6	High positive	High positive

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 as an online program (http://www.matforsk.no/ola/fisher.htm). For all tests, P-values ≤ 0.05 were considered significant. Univariate OLR was used to characterise the relationship between serological score and six factors of interest – breed; age; gender; number of cats per household; whether the cat was housed exclusively indoors, or both indoors and outdoors; and the health status of the cat at the time of blood collection.

Selected factors of interest were further examined independently. Median scores of various subsets of the sample populations (domestic versus purebred, cats younger than 2 years-of-age versus cats greater than 2 years-of-age, male versus female, single cat households versus multiple cat households, exclusively indoor cats versus indoor/outdoor cats, and health status group 1 versus group 2) were compared by Mann Whitney *U* tests. Median scores of cats with and without a recorded history of clinical signs associated with primary FeCV infection were also compared by Mann Whitney *U* tests. Kruskal-Wallis analysis was used to identify differences in median scores of cats of different breeds. Fisher's exact test was used to compare the seroprevalence between cats less than 2 years-of-age and greater than 2 years-of-age, male and female cats, single cat households and multiple cat households, indoor only and indoor/outdoor cats, and health status of group 1 and group 2 cats, as well as the seroprevalence of cats with and without a recorded history of clinical signs associated with primary FeCV infection. The median age of seropositive and seronegative cats, and the median age of health status group 1 and group 2 cats were each compared by Mann Whitney *U* tests. Confidence intervals for the median were determined by one-sample Sign tests. The median score of cats with immunohisto-

chemically confirmed FIP was compared to the overall median for the owned cat sample population by a Mann Whitney *U* test.

Results

Owned cat population

Serum samples from 306 owned cats were tested for antibodies directed against FCoV. The seroprevalence of FCoV infection in these cats was 34% (104/306). The overall median Immunocomb® score across the owned cat sample population was 1. The distribution of Immunocomb® scores in the owned cat sample population is illustrated in Figures 1 and 2.

Breed

Median scores were calculated for the 10 breeds that were represented by a minimum of three cats, and ranged from 1 (Devon Rex, DSH, Persian and Siamese) to 4.5 (Russian Blue and Ragdoll, Figure 3). There were significant differences between the median Immunocomb® scores for different breeds in the owned cat population and the overall median. Specifically, the median Immunocomb® score of DSH, Persians, Siamese and Devon Rex (1) was significantly lower than that of Burmese, BSH, Ragdoll, Russian Blue, Birman and Abyssinian cats ($P < 0.0005$). The median Immunocomb® score of domestic cats (1) was significantly lower than that of purebred cats (2; $P = 0.01$).

Age

The distribution of median Immunocomb® scores by age (Figure 4) was roughly bimodal with one major peak (median Immunocomb® score 3) recorded in kittens less than 1 year-of-age. Median values then progressively decreased as the age of cats increased, until a second smaller peak (representing the median Immunocomb® score 2) in cats 11 and 12 years-of-age.

The median age of seropositive cats (5 years) was significantly lower than seronegative cats (8 years; $P = 0.02$). The median Immunocomb® score of cats less than 2 years-of-age (2) was significantly higher than in cats greater than 2 years-of-age (1; $P = 0.03$). Similarly, the seroprevalence of FCoV infection of cats less than 2 years-of-age (44.3%) was higher than cats greater than 2-years-of-age (31.6%), however this was not statistically significant ($P = 0.07$). The difference in median scores became more marked when considering only purebred cats in these two age categories (median Immunocomb® scores 3 and 1, respectively; $P = 0.02$). Conversely, there was no distinction between median Immunocomb® scores for domestic cats divided into these age categories (median Immunocomb® scores 1 for both; $P = 0.79$).

Gender

Serum samples from 124 female (41%) and 182 male (59%) cats were tested for FCoV antibodies. The median Immunocomb® score of female cats (1) was not significantly different from that of male cats (1; $P = 0.26$). The seroprevalence of infection of female cats (31%) was marginally lower than male cats (36%), however this was not statistically significant ($P = 0.46$). Overall, there was no significant association between the gender of tested cats and Immunocomb® score ($P = 0.91$).

Number of cats per household

A strong positive relationship was observed between the median Immunocomb® score of owned cats and the total number of cats per household at the time of blood collection (Figure 5; $P < 0.0005$). The median Immunocomb® score of cats living in single cat households (1) was significantly lower than the median Immunocomb® score of cats living in households with two or

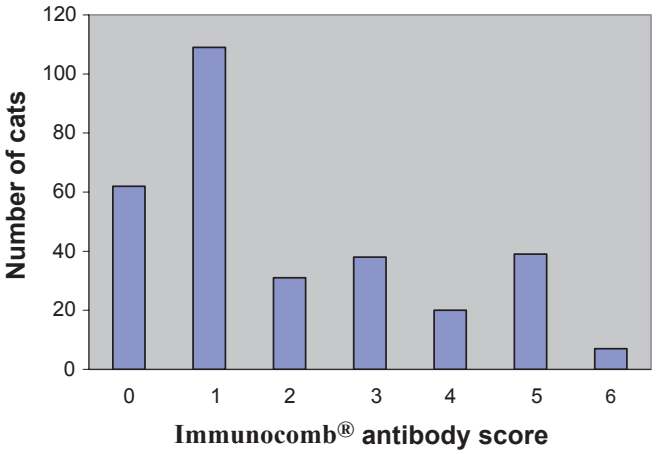

Figure 1. The distribution of Feline Coronavirus Immunocomb® antibody scores in the owned cat sample population.

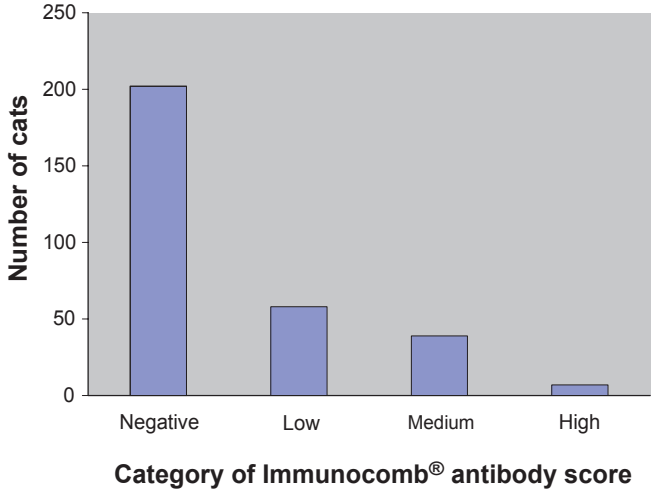

Figure 2. Distribution of the Feline Coronavirus Immunocomb® antibody scores by categories in the owned cat sample population.

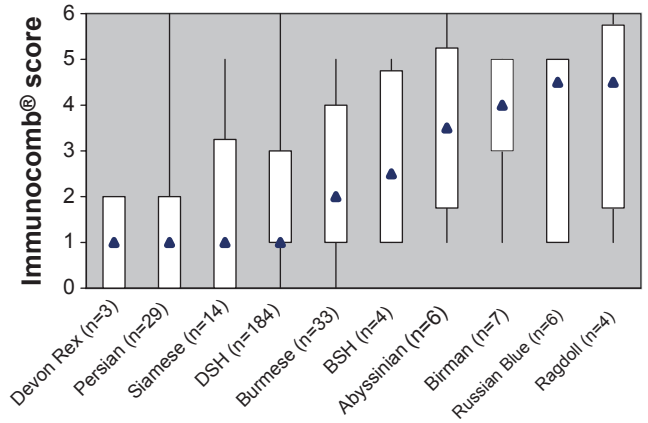

Figure 3. Boxplot of the Feline Coronavirus Immunocomb® antibody scores by breed of cat (median = triangle; interquartile range = box; range = line).

more cats (2; P = 0.003). The seroprevalence of FCoV infection in single cat households(33/140; 24%) was likewise significantly lower than in multiple cat households (59/135; 44%; P = 0.0005).

Indoor and/or outdoor status

Cats were classified as indoor only (n = 26) or indoor/outdoor (n = 75), on the basis of the available information, when provided, from the clinical record. The median Immunocomb® score of exclusively indoor cats (3) was higher than in cats allowed access to outdoors (1), however this was not statistically significant (P = 0.12). The seroprevalence of FCoV infection in exclusively indoor cats (54%) was also higher than the seroprevalence in indoor/outdoor cats (36%) but this was not statistical significant (P = 0.16).

Health status at the time of blood collection

Cats were classified as group one ('healthy'; n = 160; 52%) or group two ('sick'; n = 146; 48%) based on health status at the time of blood collection. The median age of 'healthy' cats (5 years) was significantly less than the median age of 'sick' cats (9 years; P < 0.0005). The seroprevalence of FCoV infection of 'healthy' cats (39%) was significantly greater than that of 'sick' cats (28%; P = 0.04). However, the median Immunocomb® scores of 'healthy' (1) and 'sick' (1) cats were not significantly different (P = 0.12) and, overall, classification of cats into these two groups was not significantly associated with Immunocomb® score (P = 0.84).

Disease associations

The median Immunocomb® scores of tested cats with a recorded history of one or more of the following clinical signs – diarrhoea, pyrexia, abdominal pain and vomiting – which have been associated with primary FeCV infection, were compared to the median scores of cats without any of the above clinical signs. The median Immunocomb® scores of cats with and without a recorded history of presumptive viral gastroenteritis were also compared. There was no significant difference between the median Immunocomb® scores of cats with and without a recorded history of any of these clinical signs. Similarly, there was no significant difference between the seroprevalence of FCoV infection in cats with and without a known history of any of these clinical signs or infections.

Cats with immunohistochemically confirmed FIP

Fifteen cats with immunohistochemically confirmed FIP were tested for the presence of antibodies directed against FCoV. Twelve of these cats (80%) were purebreds (five BSH, five Burmese, one Scottish shorthair and one Cornish Rex) while three were DSH. The age range of the 15 cats was 4 months to 8 years; the age of one tested cat was unknown. There were ten males and five female cats. All tested cats were seropositive. The range of Immunocomb® scores was 4 to 6. The median Immunocomb® score of cats with immunohistochemically confirmed FIP (5) was significantly higher than the median Immunocomb® score of cats in the owned cat sample population (1; P = 0.0004).

Feral cats

Blood samples from 49 feral cats trapped between July and October 2003 were tested for FCoV antibodies. The estimated age of these cats ranged from 8 weeks to 5 years. All were DSH. All 49 cats tested were seronegative. The range of Immunocomb® scores was 0 to 2.

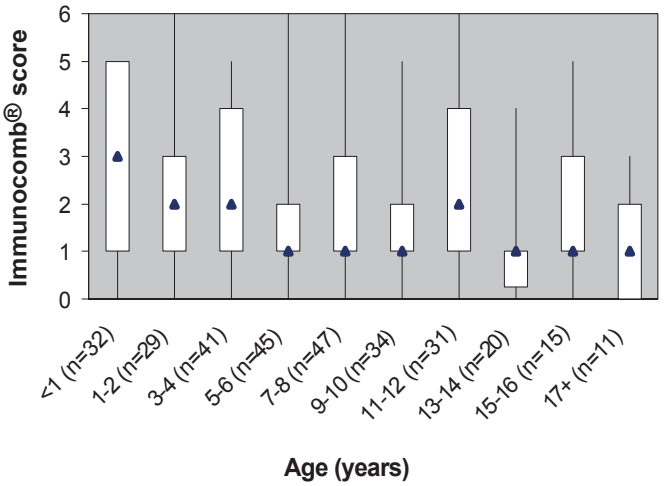

Figure 4: Boxplot of the distribution of Feline Coronavirus Immunocomb® antibody scores by age group (median = triangle; interquartile range = box; range = line).

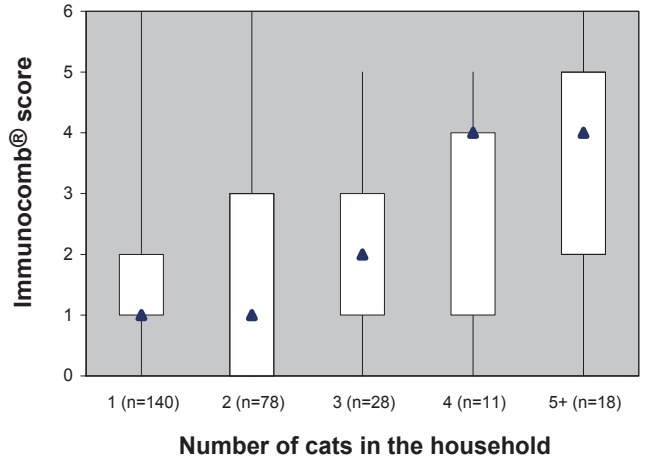

Figure 5. Boxplot of Feline Coronavirus Immunocomb® antibody score versus total number of cats in the household at the time of blood collection (median = triangle; interquartile range = box; range = line).

Discussion

This study represents the first prospective FCoV seroprevalence study of owned and feral cats in Sydney, Australia and has made several contributions to the understanding of FCoV epidemiology. The most novel finding was the highly significant association between breed and FCoV Immunocomb® score in owned cats. Previous published studies[6,9,10] have recognised a lower seroprevalence in domestic than in purebred cats. Our analysis extended to a variety of purebred cats and detected a conspicuous distinction between breeds that has not been reported previously. The significantly lower median Immunocomb® score of DSH, Persian, Siamese and Devon Rex cats compared with that of Burmese, BSH, Ragdoll, Russian Blue, Birman and Abyssinian cats supports the theory that there are true breed differences in susceptibility to FCoV infection that cannot be attributed simply to a population-dense cattery background facilitating FCoV transmission. This study supports and extends the findings of a

retrospective study by the authors and opens the door to investigations into breed related variation in FCoV susceptibility and immune responsiveness.

The overall seroprevalence of FCoV infection in our client-owned cat population was 34%. This is higher than previously published estimates of seroprevalence for overseas owned cat populations, the majority of which approximate 20 to 25%.[1,2,4,9,10] However, the careful construction of the sample population and the total of 306 cats sampled (sufficient to estimate prevalence of 34% with allowable error of 5% and level of confidence of 95%) in this study ensured that results were likely to reflect the true seroprevalence in the target owned cat hospital population of inner city Sydney from which tested cats were sourced. This study design distinguishes the present work from many previously published surveys of FCoV seroprevalence, which were either less selective in composing their sample population, or merely sampled a 'convenience' population without first establishing selection criteria.

The Immunocomb® FCoV antibody test kit was selected for use in this study for two reasons: i) the favourable correlation with the gold standard test (IFAT)[22] and ii) the ease of application. The suggested interpretation of serological results provided by the manufacturer was modified, based on Addie's[21] findings when evaluating this and other tests in comparison with IFAT.[22] Using these modifications, Addie concluded that the Immunocomb® compared favourably with the IFAT, with a sensitivity of 95% and a specificity of 83%.[22] Using the modified interpretations, cats with scores of 1 and 2 were considered seronegative, although the presence of low levels of antibodies in these cats was indicative of previous exposure to FCoV. Had these scores been considered positive, 79% of the owned cat population would have had serological evidence of previous exposure to FCoV. Whether the true seroprevalence of FCoV infection in our sample population is 34% or 79%, this observation further illustrates that the vast majority of FCoV infections do not give rise to FIP.

The seroprevalence of FCoV infection in single cat households was significantly lower than in multiple cat households, consistent with other seroprevalence studies.[6,10,23] It is likely that this reflects facilitated transmission of faecal-oral pathogens such as FCoV within environments where multiple cats use the same litter tray. FCoVs are highly contagious, and within groups of cats it has been demonstrated that almost 100% of susceptible individuals will become infected following exposure.[16,24] Importantly, previous FCoV infection does not impart protection from re-infection. When the improbability of exactly synchronising infections and time of shedding periods between cats in a household is also considered, the likelihood of continual re-infection of cats housed together is obvious. It has been proposed that urbanisation and an associated increasing stocking density of cats over the past 50 years is one major factor to explain the sudden emergence of FIP in the 1960's and its persistence since then.[8] Breed related differences in FCoV susceptibility adds further to this scenario.

While the observations in the present study seem conceptually consistent with epidemiological knowledge of FCoV infections, they contradict the findings of two recent studies of cats in the UK.[9,10] Cave and colleagues[10] reported a seroprevalence of 20% in free-ranging cats, almost twice as high as in indoor only cats (11%). Similarly, Muirden[9] found that FCoV seroprevalence in feral cats (41%) was significantly higher than in tame cats (21%). The discrepancy between these results and those of the present project may be attributable to significant environmental differences between Australia and the UK. The Australian climate is considerably warmer and drier than in the UK, and these harsher conditions may inactivate FCoV in the environment more rapidly, decreasing the length of the infectious period for cats with access to the outdoors. Another possibility is that the population density may have been lower in the present study, decreasing the potential for faeco-oral transmission. Equally the feral population may simply represent a 'multicat household' with low viral burden as seen in 56% of seronegative multiple cat households in the owned cat population.

Muirden's[9] findings stimulated our investigation of feral cats as a high prevalence of seropositivity in this cohort would impact on the control of FCoV infection, with such cats representing a potential source of FCoV for owned cats given access to the outdoors. However, the seroprevalence in the colony of feral cats studied in this project was 0%. Twenty-five cats (51.0%) had evidence of low levels of FCoV antibody (Immunocomb® scores of 1 or 2). As the reported specificity of the Immunocomb® test is 83%,[22] it was unlikely that all these results were false positives; rather, it is probable that some proportion of these cats had low circulating antibody levels. Thus it is likely that these cats had been exposed previously to the virus. The negligible seroprevalence was therefore not a function of absence of exposure but rather attributable to reduced or absent viral loads. By defecating outside over a considerable territory, with adequate burial of the stool by soil, the likelihood of exposure of feral cats to the faeces of other cats, and repeated exposure to their own faeces, is reduced. Consequently, the risk of continual re-infection is decreased, with a corresponding reduction in viral load and more likely eventual elimination of infection. The discordance between our results and those of Muirden[9] may, alternatively, reflect a difference in lifestyle of feral cat populations between the two countries. The colder UK climate may encourage colonies of cats to cluster together in warm niches. In the future it would be of interest to establish and compare the seroprevalence of FCoV infection in other feral cat colonies in Australia, particularly within suburban residential areas in which feral cats are generally in greater contact with each other and with outdoor owned cats.

The roughly bimodal age distribution of median antibody scores in the owned cat population was an interesting phenomenon that has not been reported previously. This distribution is somewhat reminiscent of the bimodal age distribution of symptomatic FIP, in which the majority of cases occur in cats less than 2-years-old, but with a second smaller peak in cats between 14 and 15 years-of-age.[25] In the present study, the median antibody score of cats less than 2 years-of-age was significantly higher than in cats greater than 2 years-of-age. This may indicate that the consistently identified predisposition of cats less than 2 years-of-age to FIP may be, at least partly, a reflection of higher viral loads in cats of these age groups. Alternatively, the higher FCoV seroprevalence in cats less than 2 years-of-age may reflect most cats being exposed to FCoV while young, with those most susceptible to FIP succumbing during or soon after their first significant infection.

This second peak in incidence of FIP has been attributed to an age-associated decline in cell-mediated immunity.[25] It is unclear what clinical significance, if any, should be placed on the later peaks of median antibody score identified in the present study in cats aged 11, 12, 15 and 16-years-old. However, these peaks in median antibody score were observed independently of other factors (breed and number of cats per household). It is unclear whether these peaks reflected re-infection of older cats, or

whether they represented a recrudescence of a previous (possibly life-long) FCoV infection that had been kept in check by the immune system until this time. An immunological lapse could potentially be related to a decrease in cell-mediated immunity in some older cats, immunosuppression due to concurrent disease (which may be more common in older cats), or the administration of immunosuppressive chemotherapy. Such an immunological lapse could facilitate viral replication with consequent increase in antibody titres in affected cats. Serological testing of a greater number of cats 11 years-of-age and older and serial monitoring of individual cats is necessary to determine whether this observation is consistently reproducible.

The possible influence of gender on serological score was of interest in this study, based on observations in a retrospective cohort of FIP cases where male cats were over-represented.[26] In the current study, no significant association between either median antibody score or seroprevalence of FCoV infection and gender was identified, similar to results of other studies.[9,10] An equal number of male and female cats were sought for testing, however this could not be achieved within the limited time frame and defined selection constraints of this project. The absence of an identifiable association between gender and infection status suggests that this was unlikely to have had a significant impact on the overall results of the study.

The seroprevalence of FCoV infection of cats classified as 'sick' was significantly lower than the seroprevalence of 'healthy' cats. This may be partially related to suppression of humoral immunity in 'sick' cats due to concurrent disease/s and/or administration of immunosuppressive agents. Examination of the age of cats in each of these two groups revealed that the median age of 'sick' cats (9 years) was significantly higher than the median age of 'healthy' cats (5 years). It is therefore probable that the difference in seroprevalence is actually a reflection of the greater proportion of young cats, including many with higher antibody scores within the 'healthy' cohort. The absence of any association between health status at the time of blood collection and antibody score was consistent with results from overseas studies[10] and suggested that FCoV infection was not significantly associated with disease (other than FIP) in cats.

The median antibody score of cats with FIP was significantly higher than the median antibody score of cats in the general pet cat population in this study. While cats with FIP had medium or high antibody scores (4, 5 or 6) these titres per se were by no means diagnostic of FIP, for similar antibody scores were also observed in 22% of the general owned cat population. This clearly demonstrates that definitive serodiagnosis of FIP is not possible. The usual diagnostic limitations of FCoV serology apply to the Immunocomb® test, and it should be remembered that FCoV serology can neither confirm nor disprove a diagnosis of FIP.

Overall, the present study estimated the seroprevalence of FCoV infection within the defined owned cat population of Sydney's inner city to be 34%, which is considerably higher than overseas estimates. The sample cohort is likely to be representative of the hospital population from which tested cats were sourced due to the careful construction of the sample population. This estimation of seroprevalence was contrasted by a seroprevalence of 0% in a Sydney feral cat population. It seems likely that an intermediate seroprevalence would be expected in suburban parts of Sydney, but more work is required to establish this. The major determinants of antibody score of owned cats identified in this study were breed, age and the number of cats per household. These findings emphasise that, if serology is being used as an aid to the diagnosis of FIP in owned cats, the breed, age and environment of the cat must be considered when interpreting serological results. While the median antibody score of cats with confirmed FIP was significantly higher than that of the overall owned cat population, these results failed to provide a definitive diagnosis, being also observed in many healthy cats. The diagnostic limitations of FCoV serology should always be remembered when using these tests in cats with suspected FIP.

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