The relationship between the Feline Coronavirus antibody titre and the age, breed, gender and health status of Australian cats

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Objectives To investigate the relationship between Feline Coronavirus (FCoV) antibody titres and age, breed, gender and health status of Australian cats

Design Retrospective study

Procedure Results from two serological tests that measure FCoV antibody levels, the Coronase test and the 7B Feline Infectious Peritonitis (FIP) test, were recorded over a 2-year period, with patient signalment, history, presenting complaint and the reason for ordering the test (as available). Results from each antibody test were related to four explanatory variables (breed, age, gender and health status at the time of blood collection) using univariate ordinal logistic regression analyses, Mann Whitney U tests, one-sample sign tests or Kruskal-Wallis analyses, as appropriate.

Results Results from 637 Coronase and 191 7B FIP antibody tests were recorded. There were significant differences in median Coronase antibody titres between breeds of cats (P < 0.0005). Specifically, the median Coronase antibody titres of Siamese, Persians, Domestic Shorthairs and Bengal cats (100) were significantly lower than that of British Shorthairs, Cornish Rex and Burmese cats (400, P < 0.0005). There was no statistical relationship between the Coronase or 7B FIP antibody titres and age, gender or overall health status, even when considering only those cats in which clinical signs suggestive of FIP were present.

Conclusion This study reinforces the complexity of interpreting serological tests for FCoV in both healthy cats and patients with signs compatible with FIP. Unique to this study is the detection of a significant relationship between breed and median FCoV antibody titre. This supports the theory that breed related differences exist in response to FCoV infection. The distribution of median Coronase antibody titres by breed was very similar to the pattern of breed predisposition to FIP recently reported in Sydney.1

FIP is a progressive, debilitating, ultimately fatal systemic disease of domestic and wild felids caused by infection with mutant variants of the ubiquitous FeCV. These mutant variants are commonly referred to as feline infectious peritonitis virus or FIPV. The enigmatic nature of FIPV infection, coupled with the uniqueness and gravity of the disease, has generated a great deal of interest amongst researchers. Despite considerable advances in our understanding, much remains unknown and FIP continues to be one of the most significant infectious diseases of cats. One of the most challenging practical aspects of FIP is procuring a definitive ante-mortem diagnosis. A wide range of tests, each with limitations, has been developed to facilitate a definitive diagnosis of FIP. Consideration of these limitations is imperative for the proper use and interpretation of these test results.

Since the development of the first technique to detect FCoV serum antibody titres in 1976,2 interpretation of FCoV serology has been a controversial topic in feline medicine. Serum antibody tests are considered by some workers to be helpful in supporting a clinical diagnosis of FIP3 although they must be considered together with the patient’s history and other clinicopathological findings and can never be used alone.4,5 While an indirect immunofluorescent antibody titre for FCoV of 1:1600 is claimed to have a positive and negative predictive value for FIP of 0.94 and 0.88 respectively, antibody titres below this end titre are unreliable and fraught with interpretative difficulties.6 Furthermore, qualitative tests that simply detect the presence or absence of FCoV antibodies are not helpful in diagnosing FIP. Despite this, recommendations for the use of serology and the management of seropositive cats have varied. The current view, however, is that the euthanasia of cats on the basis of serological tests alone, irrespective of the magnitude of the titre, can never be justified.6,7 FCoV serology can, however, provide valuable information about the dynamics of infection within groups of cats, either as part of control programs within breeding catteries and shelters,8 or when used to conduct epidemiological studies within wider feline populations.9 While FCoV serology is unable to predict which cats will go on to develop FIP, it is accepted that the magnitude of the FCoV antibody titre reflects the viral load within a given host.10,11 Although this is not a direct predictive relationship,12,13 some researchers have speculated that certain cats with persistent and/or high FCoV antibody titres, given the right host susceptibility, may be at a greater risk of developing FIP.

A range of serological tests used to measure FCoV antibody titres is available and marketed for veterinary use. The two most common uses for FCoV serology are i) as an aid to the diagnosis of FIP in cats with history and signs consistent with the disease; and ii) to determine if a presently healthy cat has been exposed to FCoV. The latter is most commonly requested as part of a

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BSH British Shorthair cats
DSH Domestic Shorthair cats
ELISA Enzyme linked immunosorbent assay
FCoV Feline Coronaviruses
FeCV Feline enteric coronavirus
FIP Feline Infectious Peritonitis
FIPV Feline Infectious Peritonitis virus
OLR Ordinal logistic regression
PCR Polymerase chain reaction
FCoV cattery control program or in a household of cats where one cat has died of FIP. The greatest difficulty for the veterinarian then lies in interpreting the test results. Specifically, the absence of published peer-reviewed data concerning the pathogenic role of mutated genes and proteins detectable by serology, highlights the need to exercise care to avoid over interpretation of test results.

The majority of commercial FCoV serological testing in Australia is conducted by VETPATH®, a private veterinary pathology laboratory in Perth, Western Australia. Until recently, two distinct FCoV serological tests were performed in this laboratory – the Coronase test and the 7B FIP test. These serological tests have recently been replaced by the indirect immunofluorescent antibody test, which is considered to be the ‘gold standard’ test for the measurement of FCoV antibodies worldwide.14

The Coronase test is an ELISA that detects antibodies directed against FCoV. Seropositivity reflects past exposure to one or more FCoV (which may be either FeCV or FIPV) or, less commonly, another closely related coronavirus, for example canine coronavirus or transmissible gastroenteritis virus of pigs. The 7B FIP test, also based on an ELISA technique, differs from other available FCoV antibody assays in detecting antibodies directed against one specific protein (the 7B protein). The 7B-FIP test is based on the incorrect premise that the 7B protein is coded for by a region of the FCoV genome unique to FIPV strains, and that only antibodies directed against FIPV are detected. Vennema and colleagues15 used PCR and sequencing to compare the genomes of FeCV and FIPV strains, but could not identify consistent distinguishing regions. Furthermore, Herrewegh and colleagues16 reported that deletions in the 7B region did not occur in all isolates of FeCV studied by sequence analysis, which would indicate a potential for false positive results of a test based on this part of the FCoV genome. Subsequently, Kennedy and colleagues17 described the application of a PCR protocol specific for the 7B region, and reported that this procedure was unable to discriminate consistently between FeCV and FIPV strains.

The objective of this study was to investigate the relationship between FCoV antibody titres and the age, breed, gender and health status of Australian cats. This paper is the second in a series of papers on FIP and FCoV infection in Australian cats. The third and fourth papers in this series are prospective sero-prevalence studies in owned pet cats and cattery-confined cats in Australia.

Materials and methods

Data collection

Results from two serological tests measuring FCoV antibody levels, the Coronase test and the 7B FIP test, performed at VETPATH® over a 2-year period (June 2001 to June 2003) were recorded in an Excel® spreadsheet, in conjunction with details of age, breed, gender, history, presenting complaint and the reason for ordering the test, provided by the referring veterinarian on the request form. The reason for requesting each test (where known) was used to divide samples into two separate groups for independent evaluation: i) tests used as an aid to the diagnosis of FIP in cats with a potentially compatible history and/or physical findings; and ii) tests used to determine if a presently healthy cat(s) had been exposed to FCoV, (for example as part of routine cattery management programs, or in a multicasual household in which one cat had, or was suspected of having, FIP). Additionally, results from cats with one or more physical or biochemical finding suggestive of FIP (ascites, pleural effusion, persistent pyrexia, uveitis, neurological signs, hyperglobulinaemia, weight loss) were further divided into groups for additional assessment.

Data analysis

All statistical analyses were performed using a commercial statistical software package (Minitab® v.13.32 for Windows). For all tests, P-values £ 0.05 were considered significant. Results from each antibody test were related to four explanatory variables (age, breed, gender and health status at the time of blood collection) by means of two separate univariate OLR analyses.

Selected factors for both tests were further examined independently. The median titres of cats with clinical signs suggestive of FIP (group 1) and clinically healthy cats (group 2) within each breed were compared by Mann Whitney U tests. Median titres of domestic versus purebred, and male versus female cats, for both tests, and < 2 years-of-age and > 2 years-of-age for the Coronase test, were also compared by Mann Whitney U tests. One-sample sign tests were used to identify differences between the median titres of cats with certain physical findings consistent with FIP and the overall median for the Coronase test. Similarly, Mann-Whitney U tests were used to compare median titres of cats with and without a recorded history of certain clinical signs. Kruskal-Wallis analyses were used to identify significant differences in median titres within each breed for each test. The correlation between Coronase and 7B FIP test results for blood samples that were tested concurrently was calculated to determine the degree of association between the two tests.

Results

Coronase antibody test results

Results from 637 Coronase antibody tests performed at VETPATH® on samples sent from all states and territories of Australia between June 2001 and June 2003 were recorded. Results were reported as one of the following antibody titres (noted here and subsequently as a reciprocal titre): 0, 25, 100, 400, 800 or 1600. The overall median Coronase antibody titre was 100 (range 0 to 1600).

Breed

The distribution of median Coronase antibody titres for individual cat breeds (represented by a minimum of eight cats) is shown in Figure 1. There was a wide range of median antibody titres in these 12 breeds, from the lowest median antibody titre of 62.5 in the Siamese and Burmilla to the highest median antibody titre of 400 in the Burmese, Cornish Rex and BSH. There were significant differences in median Coronase antibody titres of these 12 breeds (P < 0.0005). The median antibody titres of Siamese, Persians, DSH and Bengal cats (100) were significantly lower than that of BSH, Cornish Rex and Burmese cats (400; P < 0.0001). Similarly using geometric means, Siamese, Persians, DSH and Bengal cats (mean = 43) had significantly lower antibody titres than BSH, Cornish Rex, Birman and Burmese cats (mean = 138; Figure 2).

Age

No significant association between the age of cats and the Coronase antibody titre was identified (P = 0.116), although there was a trend towards an inverse relationship between these two variables. The distribution of median Coronase antibody titres by age is illustrated in Figure 3.
Coronase test results from 332 female and 275 male cats were recorded, while the gender of 30 tested cats was indeterminate. The median antibody titre of female (100) and male cats (100) was identical and therefore no significant association between the gender and Coronase antibody titre was identified (P = 0.240).

Health status at the time of blood collection
Coronase antibody test results were divided into two separate groups according to the reported health status of the cat at blood collection. There were 133 cats tested in group 1 (those with suspected FIP) while 164 cats were tested in group 2 (clinically healthy).

The median Coronase antibody titre for suspect FIP cats (100) was identical to the median Coronase antibody titre of asymptomatic cats (100; P = 0.2825). There were no significant differences in the median titres of group 1 and group 2 cats when divided into individual breeds, with the exception of Persian cats for which the median Coronase antibody titre of group 1 cats (12.5) was significantly lower than for group 2 cats (250; Table 1).

There was no significant difference between the median titres of group 2 domestic and purebred cats (P = 0.7573). Overall, no significant association between the health status of cats and the Coronase antibody titre could be identified (P = 0.516).

Clinical findings associated with FIP
The median Coronase antibody titres of cats with one or more of the physical or biochemical abnormalities suggestive of FIP as stated in the methods, were compared to the overall median Coronase antibody titre. There was no significant difference between the median Coronase antibody titres of cats for any of the physical or biochemical findings examined and the overall median (Table 2).

7B FIP antibody test results
Results from 191 7B FIP antibody tests performed at VETPATH® between June 2001 and June 2003 were recorded. The results of this 7B FIP test were reported by VETPATH® as one of the following reciprocal antibody titres: 0, 40, 80, 160, 320 or 640. The overall median 7B FIP antibody test titre was 40 (range 0 to 640).

Breed
The range of 7B FIP antibody titres of the breeds represented by six or more cats is shown in Figure 4. There was no significant difference in the median 7B FIP antibody titres of these seven breeds (P = 0.124). The median 7B FIP antibody titre (0) of domestic cats (n=49) was not significantly different from the median 7B FIP antibody titre (40) of purebred cats (n = 123; P=0.6661). Overall, a significant relationship between 7B FIP antibody titre and breed was not identified (P = 0.797).

Age
No significant association between age of tested cats and 7B FIP antibody titre was identified (P = 0.263).

Gender
The 7B FIP test results were recorded for 102 female and 72 male cats while the gender of 17 tested cats was not recorded. The median 7B FIP antibody titre of female cats (40) was not significantly different from the median 7B FIP antibody titre
groups on the basis of the reported health status of the cat at blood collection. There were 46 cats tested in group 1 (suspected FIP) while 44 cats were tested in group 2 (clinically healthy).

The median 7B FIP antibody titres of group 1 (0) and group 2 cats (40) were not significantly different (P = 0.9371). The only breeds represented in sufficient numbers in both groups to facilitate this comparison were the Burmese and DSH cats. There were no significant differences in the median titres of Burmese and DSH group 1 and group 2 cats. Overall, no significant association between the health status of the cat at the time of blood collection and the 7B FIP antibody titre was identified (P = 0.684).

Clinical findings associated with FIP
The median 7B FIP antibody titres of cats with one or more of the physical or biochemical abnormalities stated in the methods, were compared to test results from cats without these reported abnormalities. There was no significant difference between the median 7B FIP antibody titres of cats with and without any of these clinical findings.

Correlation between Coronase and 7B FIP test results
Blood samples from 75 cats were submitted for concurrent testing with the Coronase and 7B FIP tests. Results from the two tests were positively correlated (P=0.028), indicating that high titres in one test were associated with high titres in the other test, and conversely that low titres in one test were associated with low titres in the other test.

Discussion
A major finding from this serosurvey was the lack of discernable association between the results of serological tests for FCoV and the health status of a given cat. This observation highlights the difficulty in interpreting coronavirus serology, both in patients suspected of having FIP and in healthy cats tested as part of a screening program. Another major finding was the significant relationship between breed and FCoV antibody titre. This finding has not been detected previously, presumably because previous serosurveys have not focused on breed per se. Most notable was the significantly higher Coronase antibody titres observed in BSH, Burmese and Cornish Rex cats. These three breeds were significantly over-represented in our recent retrospective study of FIP cases in Australia.1 This supports the theory that there are critical breed-related differences in the immune response to FCoV. This observation may lead to investigations of breed-related variations in immune response to FCoV.

The substantial number of results from purebred cats in this study facilitated a more detailed statistical analysis and comparison between breeds that was not feasible in the prospective study conducted concurrently by the authors. Overall, a highly significant relationship between the breed of cat tested and Coronase titres was identified. Considerable variation in the median Coronase antibody titres of different breeds was apparent. Consistent with results from previous seroprevalence studies,10,18,19 the median Coronase antibody titre of domestic cats was lower than for purebred cats. However, when breeds were considered individually, the median antibody titres of Siamese and Persian cats were lower than for domestic cats. Of particular interest was the observation that the distribution of median Coronase antibody titres by breed was very similar to the pattern of breed predisposition to FIP in Sydney recently.
identified by Norris and colleagues.1 While it could be argued that the higher median titres of over-represented breeds is a consequence of more cats of these breeds having symptomatic FIP, the conservation of these trends irrespective of the health status of cats argues against this proposition. When considered together with findings from other components of this project, it seems much more likely that there are true genetic differences in susceptibility to FCoV infections between breeds, presumably related to differences in immunological response. Breed related predispositions to infectious agents, for example cryptococcosis, have been reported previously.20

While the explanation for the consistently higher antibody titres of certain breeds is not known definitively, it may be a function of: i) a less effective immunological response to FCoV infection, resulting in greater difficulty in constraining or eliminating the infection, leading to increased viral replication, longer periods of infection and greater viral loads; and/or ii) the generation of a more intense humoral immune response to FCoV infection in these breeds than in other breeds. The production of a humoral response to FCoV infection in the absence of an effective cell-mediated immune response is ineffective in controlling FCoV infection, and may enhance uptake of FCoV into macrophages. The identification of parallels between breed susceptibility to FIP and high antibody titres suggests that predisposition to disease is at least partly correlated with the existence of greater viral loads in these breeds, with a corresponding increased chance of FIPV arising by mutation in vivo. Although the actual scenario is likely to be more complex, it is possible that concerted efforts to decrease viral loads in susceptible breeds may yield a lower incidence of FIP.

Breed-associated variation in the immune response to FCoV is likely to be an important factor determining the susceptibility or resistance of certain breeds to FIP. For example, differences in the function of T-lymphocyte subpopulations and their cytokines may exist between individuals. Further characterisation of the immune response in cats with FIP is required to investigate possible breed specific aspects of the immunopathogenesis of FCoV infection and FIP.

No significant association between the gender of tested cats and either the Coronase or 7B FIP test titres was identified in this study, which is comparable to results from other studies.18,21 This observation suggests that any gender-related differences do not have a significant impact on transmission of FCoV.

A trend towards an inverse relationship between Coronase titre and age was identified in this component of the study. This is similar to reported findings of others.19 Cats are commonly infected with FCoV at a young age. This may reflect a combination of the cat-dense environments from which most kittens, regardless of breed, are acquired, as well as the general immaturity of young animals, which predisposes them to many infectious diseases. Most infected cats shed FCoV for less than 1 year after infection.4 They are therefore able to reduce viral loads over time, particularly if they are rehoused in single or two-cat households where they will be much less likely to be continually re-infected by other shedding cats, especially young cats.

The high proportion of test results available from cats with suspected FIP was used to investigate the value of FCoV serological antibody tests for the diagnosis of FIP. One of the most significant findings of this study was the absence of an identifiable association between the health status of tested cats at the time of blood collection and either Coronase or 7B FIP antibody titres. The median Coronase antibody titre of cats for which FIP was considered to be a differential diagnosis was not significantly different from the median titre of presently healthy cats, for which the Coronase test was used to determine previous exposure to FCoV. The median 7B FIP antibody titre of cats suspected of having FIP was, ironically, lower than the median titre of healthy cats, although this difference was not significant.

The most obvious limitation of this study was the lack of follow-up information regarding the outcome of disease in those cats tested due to suspected FIP. It is not known whether these cats recovered, died of another disease that resembled FIP clinically, or whether they indeed had histologically confirmed FIP. It is therefore possible that the inclusion of an indeterminate proportion of cats which did not have FIP within group 1 decreased the overall median of group 1 cats to a lower value than would have been recorded had only cats with confirmed FIP been included. However, when both Coronase and 7B FIP antibody titres from cats with specific clinical findings commonly associated with FIP were examined individually, there was likewise no significant distinction between the median titres of cats with any of these clinical findings and the overall median antibody titre for either serological test. The clinical findings commonly associated with FIP are by no means pathognomonic for the disease, and it is possible that they were associated with an alternative pathological process in some tested cats. However, when it is considered that FIP is a common cause of ascites in young cats and that most alternative causes of this are relatively easily distinguished from FIP by basic fluid analysis, a significantly higher median antibody titre of cats with this clinical sign in particular would be expected, to demonstrate any diagnostic value of serology. None however was identified.

The most commonly stated reason for testing healthy cats with either test was as part of FCoV control programs in catteries. As identified in other seroprevalence studies19, 1,22 FCoV antibody titres in cats living in catteries are often higher than in owned pet cats. It is therefore possible that the considerable proportion of cats living in catteries within the clinically healthy group (group 2) raised the overall median titre for group 2 and potentially disguised a difference in median titre. It is therefore possible that inclusion of an indeterminate fraction of cats (which FIP was considered to be a differential diagnosis) decreased the overall median of group 1 cats to a lower value than would have been recorded had only cats with confirmed FIP been included. However, when both Coronase and 7B FIP antibody titres from cats with specific clinical findings commonly associated with FIP were examined individually, there was likewise no significant distinction between the median titres of cats with any of these clinical findings and the overall median antibody titre for either serological test. The clinical findings commonly associated with FIP are by no means pathognomonic for the disease, and it is possible that they were associated with an alternative pathological process in some tested cats. However, when it is considered that FIP is a common cause of ascites in young cats and that most alternative causes of this are relatively easily distinguished from FIP by basic fluid analysis, a significantly higher median antibody titre of cats with this clinical sign in particular would be expected, to demonstrate any diagnostic value of serology. None however was identified.

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observation, with considerable implications for clinical practice. Results of this study have reinforced the limitations associated with the application of FCoV serology as a diagnostic test in individual cats suspected of having FIP. Cats for which FIP was a differential diagnosis under consideration were serologically indistinguishable from healthy cats with both the Coronase or 7B FIP test. Additionally, the entire range of possible titres for the Coronase test was recorded with similar frequencies in clinically healthy cats and suspect FIP cases, emphasising that the absolute serodiagnosis of FIP is not possible. Antibody titres for FCoV can neither confirm nor disprove a diagnosis of FIP and, when the most appropriate outcome of testing is that the result obtained will not influence a cat’s treatment or prognosis, the use of these tests in clinical practice must be questioned.

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