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Risk factors for feline infectious peritonitis in Australian cats

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ABSTRACT

Objective: To determine whether patient signalment (age, breed, sex, and neuter status) are associated with naturally occurring feline infectious peritonitis (FIP) in cats in Australia.

Design: A retrospective comparison of the signalment between cats with confirmed FIP and the general cat population.

Results: The patient signalment of 382 FIP confirmed cases were compared with the Companion Animal Register of NSW and the general cat population of Sydney. Younger cats were significantly over-represented amongst FIP cases. Domestic crossbred, Persian, and Himalayan cats were significantly under-represented in the FIP cohort while several breeds were over-represented including British Shorthair, Devon Rex, and Abyssinian. A significantly higher proportion of male cats had FIP compared to female cats.

Conclusion: This study provides further evidence that FIP is primarily a disease of young cats and that significant breed and sex predilections exist in Australia. This opens further avenues to investigate the role of genetic factors in FIP.

INTRODUCTION

Feline infectious peritonitis (FIP) is a feline coronavirus (FCoV)-induced disease of cats, characterized by immune-mediated vasculitis and development of pyogranulomatous inflammation in various tissues of the body. Clinical manifestations are generally divided into effusive (wet) or non-effusive (dry) forms.[1] Effusive FIP is characterised by fibrinous serositis with proteinaceous exudation into body cavities.[2-3] Effusion most commonly occurs in the abdominal cavity but can also occur in the thorax, pericardium, and scrotum.[4] Non-effusive FIP involves pyogranulomatous inflammation in a variety of tissues including the abdominal lymph nodes, intestines, liver, kidneys, eyes, and lungs.[2,5] In the absence of any consistently effective treatment,[4] most cases result in death or euthanasia.[6] The complex pathogenesis of FIP remains a topic of considerable interest, with recent work focusing on potential mediators of virulence and host risk factors.

The variable clinicopathologic presentation of FIP makes clinicians consider this diagnosis in a wide variety of patients however definitive diagnosis remains a challenge unless body cavity effusions and/or affected tissues are appropriately examined.[4] A positive histological diagnosis is characterised by fibrinous-granulomatous serositis, granulomatous-necrotising vasculitis and granulomatous inflammatory lesions in multiple organs.[2] Definitive diagnosis of FIP involves detection of FCoV antigen within macrophages using direct immunofluorescence (DIF) or immunohistochemistry (IHC)[7-8] and these two tests are now considered the gold standard.[4] Like IHC, DIF is highly specific (~100%)[7-11] but its sensitivity is variably reported from 95% [8,10] to as low as 57% [11] compared with histology, previously considered the gold standard.

Several risk factors have been discussed in association with the development of FIP. Disease is seen more frequently in cats less than two years-of-age,[5,12-15] residence in multi-cat environments,[12,15] and pedigree cats.[5,12-14] Male[5,13] and sexually-intact[13-14] cats are over-represented in some studies but not in others.[12] Early reports suggested that FIP had a bimodal age distribution [16] although more recent studies do not support this observation.[5,12-13]

While pedigree cats are invariably over-represented in FIP case series compared with crossbred cats,[5,12-14] the particular breeds over-represented in FIP cohorts vary between studies. A retrospective study of 42 cats from NSW, Australia with FIP confirmed histologically[5] found that British Shorthair, Burmese, Australian Mist, and Cornish Rex cats were significantly more likely to develop FIP compared with the hospital population. A study in North Carolina, USA[14] concluded that Abyssinians, Bengals, Birmans, Himalayans, Ragdolls, and Rexes (both Cornish and Devon) had a significantly higher risk, whereas Burmese, Exotic Shorthairs, Manxes, Persians, Russian Blues, and Siamese cats were not at increased risk of developing FIP. A limitation of the second study was that the criteria used to diagnose FIP were not reviewed by the authors to confirm whether the diagnosis was definitive. The variability in results amongst previous studies highlights the difficulty in conducting epidemiologic analyses into a disease that is sometimes difficult to diagnose definitively. Disparate results amongst studies could be due to sampling from differing geographic locations, different gene pools, limited sample sizes, differing methods of classifying FIP cases, or failure to identify confounding factors.

The objective of this study was to use the results of the Australia wide FIP diagnostic testing facility at the University of Sydney to determine if associations existed between naturally-occurring FIP and the age, breed, sex, and neuter status of cats in Australia.

MATERIALS AND METHODS

Selection of cases

Cases were selected by reviewing the records of all tissue and fluid samples submitted to Veterinary Pathology Diagnostic Services (VPDS), The University of Sydney, for diagnosis of suspected FIP by DIF or IHC between January 2004 and July 2011. Over the study period, VPDS was the principle laboratory in Australia offering DIF and IHC for FIP diagnosis, so submissions originated from every state and territory in Australia. Veterinarians, either directly or via their usual diagnostic laboratory, submit tissue or effusion samples from the patient based on the presenting clinical signs and the availability or accessibility of these samples for collection. DIF was performed on effusion samples while IHC was performed on formalin fixed tissue samples as described below.

A case was confirmed as FIP on the basis of either 1) Positive result for DIF together with moderate to high protein content greater than 35g/L[3] and a mixed cell population of predominantly neutrophils and macrophages with small numbers of lymphocytes[10]; or 2) Positive result for IHC with histology consistent with FIP. With the reported variable sensitivity of DIF in mind, and the biases involved in sample submission to the diagnostic laboratory, FIP negative submissions were not used as controls as part of a case-control study, as it is conceivable that some of these cases actually had FIP or other conditions where patient characteristics, especially breed, impact on disease prevalence. Instead, a control population was obtained using cat registration data and by extrapolating the results of several recent epidemiologic investigations as outlined below.

Potential risk factors

Age, breed, sex, and neuter status were recorded for all cases. Where information regarding signalment was incomplete, further enquiries were made to the clinic or laboratory of origin. Cases were excluded from analyses if further enquiries failed to determine a cat's age, breed, sex, or neuter status. Cats that had been recorded as domestic shorthair, medium hair or longhair were termed domestic crossbreds, while all other cats were classified by their breed or grouped as pedigree.

Comparison with the general cat population

The signalment of FIP cases was compared with those of the general cat population. To provide a base population for comparison of breed prevalence, breed registration data were obtained from the New South Wales (NSW) Companion Animals Register (CAR), a local statutory government-based body that administers compulsory registration of companion animals across NSW, the most populous state of Australia, and records a cumulative register of all cats residing in that state. In the absence of nation-wide information on the signalment of cats, the age and sex of cats in the general cat population were inferred from the findings of recent studies into the demographics of the Sydney cat population [17-18].

Direct immunofluorescence (DIF) for detection of FCoV in macrophages in effusions

DIF was used to identify FCoV antigen within the cytoplasm of macrophages. This was performed on cytocentrifuged effusion samples in a manner similar to that described previously[8] with some modifications. Briefly, at least two slides were prepared for each sample using 100µL of sample to which 0.2mg hyaluronidase was added (H-3506, Sigma, MO, USA) prior to cytocentrifugation to facilitate the production of a uniform monolayer of cells. Samples were cytocentrifuged using the Cytospin 2 (Shandon Southern Products, Cheshire, UK) for 5 min at 63 x *g* (750 rpm). Subsequently, slides were air-dried at room temperature for 30 min, permeabilised and fixed in 75% acetone-25% methanol for 20 min, dried in an incubator at 37°C for 30 min and then incubated in a moist chamber at 37°C for 30 min with 50µL of a fluorescein-conjugated polyclonal anti-coronavirus antibody (Cat. No

CJ-F-FIP; VMRD, Pullman, WA, USA) which detects FCoV serotypes 1 and 2. Slides were rinsed in a buffer containing Na_2CO_3, $NaCHO_3$, NaCl and deionised water (pH= 9.0) and then soaked for 10 min. Slides were mounted with an anti-fadent mounting fluid (Citifluor, Cat No. IAF1, QLD, Australia) and examined under a fluorescent microscope (model no. BX60F-3, Olympus, Tokyo, Japan) at 250 to 400 x. Positive and negative controls were run concurrently as using FIPV infected and non-infected Crandell Feline Kidney cells (Cat. No SLD-FAC-FIP and SLD-FAC-FIP2; VMRD, Pullman, WA, USA). This method has been tested on 40 histologically confirmed FIP cases and a wide variety of non-FIP diseases causing effusions in cats (controls, n=32). The specificity and sensitivity of this method in our laboratory as described is 100% and 75% respectively. Samples that clearly showed fluorescence within macrophages in two slides under 250 to 400 x magnification were considered positive, while samples were deemed negative if fluorescence within macrophages was not evident. In addition, effusion samples submitted to VPDS for diagnosis of FIP routinely underwent quantification of total protein content via refractometry, and cytological examination using a rapid modified Romanowsky stain (DiffQuik, Lab Aids, Victoria, Australia) under light microscopy.

Histology and immunohistochemistry (IHC)

Routine histological examination of haematoxylin and eosin stained sections was performed by the referring diagnostic lab, with reports sent to VPDS and/or performed at VPDS. IHC was performed following histological examination as described previously.[19] Tissue sections (4um) of formalin-fixed, paraffin-embedded tissue were mounted onto silane coated slides and dried for 24 hours at 37°C to aid tissue adherence to the slide. Slides were deparaffinized and rehydrated by submerging in 100% xylol and graded dilutions of ethanol to water. Antigen retrieval was achieved using a non-enzymatic, heat-induced method using a commercially available antigen retrieval solution (Target Retrieval Solution, 10X Concentrate, code no. S1699, DakoCytomation, Carpinteria, CA, USA) at working dilutions as per manufacturer's instructions. Slides were placed in the Dakocytomation Autostainer Plus (DakoCytomation, Carpinteria, CA, USA) where the following steps were performed. Endogenous peroxidases were blocked by incubating the slides with 0.03% hydrogen peroxide (Peroxidase Block, code K4007, DakoCytomation, Carpinteria, CA, USA) for 15 min at room temperature. Antigen detection was achieved by incubating the slides for 60 min at room temperature using a 1:1000 dilution of monoclonal antibody against nucleocapsid of FCoV (kindly donated by Professor Niels Pedersen). All slides were incubated with the secondary antibody for 30 min at room temperature (Envision Labelled Polymer-HRP Anti-mouse, code K4007, DakoCytomation, Carpinteria, CA, USA). Finally, the slides were incubated for 5 min at room temperature with 3,3'-diaminobenzidine (DAB) chromogen solution (DAB + Chromogen, code K4007, DakoCytomation, Carpinteria, CA, USA). Slides were thoroughly rinsed with DakoCytomation Tris Buffered Saline between each of the above steps. Once the Dako Autostainer had completed the DAB step, slides were rinsed in water and manually counterstained with haematoxylin, dehydrated through graded alcohol dilutions and xylol, and coverslipped prior to examination. Positive and negative controls were used in every run. Positive reagents controls ensuring that the anti-FIPV antibody was working consisted of previously confirmed cases of FIP (by histology and IHC). To evaluate non-specific staining on tissues, negative patient controls were run with each biopsy specimen. These consisted of identically prepared sections processed with the standard protocol with the exception that the anti-FCoV primary antibody was replaced with a 1:100 dilution of universal mouse serum (item number 004335, DakoCytomation, Carpinteria, CA, USA).

Statistical analysis

Chi-square analyses were conducted to determine whether the observed breed, age, sex, and neuter frequencies of cats in FIP cases differed significantly from their respective expected frequencies (Prism 5 for Windows, Version 5.03, GraphPad Software Inc., 2010). Expected breed frequencies were based on CAR registration data.[18] Only breeds with over 1000 cats registered in the CAR were included in

statistical analyses. If chi-square test was significant, the proportion of each breed in the FIP case dataset was compared with the respective proportion in the CAR registration data using z-test after Benforroni adjustment for multiple comparisons. Domestic crossbreds were excluded from analyses of observed and expected pedigree cat proportions. The median age of entire and neutered cats was compared using the Mann Whitney test. All graphs were constructed using Prism 5 for Windows (Version 5.03, GraphPad Software Inc., 2010). Results were considered significant if P <0.05.

RESULTS

Submitted samples and FIP cases

A total of 868 submissions were received by VPDS where a diagnosis of FIP was under consideration and FIP was confirmed in 382 cases (Table 1[1]). Fluid samples (n=689) were submitted for DIF while tissue samples (n=179) were submitted for IHC. Of the 689 fluid samples, 292 were considered FIP positive while 397 were negative. Of the 397 negative DIF results, FIP was considered highly unlikely in 89 (22%) samples based on the presence of bacteria, neoplastic cells or a protein content well below 35g/L. The FIP status of the remaining 308 samples could not be determined with certainty. Of 179 tissue samples, 90 were considered FIP positive by IHC while 89 were negative. Of the 89 negative samples, 84 did not have supporting histology either, making them likely true negatives. The remaining five IHC negative cases had strongly supportive histology for FIP making these likely false negatives.

Descriptive analyses

The age of FIP cases ranged from 2 months to 15 years. The majority of FIP cases were less than 1 year-of-age, with 50% under 7 months (IQR= 5 months to 1.25 years). The median age of entire cats with FIP (6 months; IQR 4 months to 8 years) was significantly lower than the median age of neutered cats with FIP (1 year; IQR 6 months to 3.25 years) ($p<0.001$). In FIP cases, the proportion of entire cats was significantly higher amongst pedigree cats than in domestic crossbred cats (OR= 2.20, CI= 1.44-2.58, $p<0.001$).

Comparison with registration and census data

A total of 439,145 cats were registered under the CAR of NSW (Table 2). The observed frequency of breeds in the FIP positive cohort differed significantly from that expected from the CAR data ($P<0.001$). The observed and expected frequencies of domestic crossbred and pedigree cats with over 1000 cats registered in NSW are shown in Figure 1. When compared to expected breed frequencies of the top ten breeds in the CAR, domestic crossbred cats were significantly under-represented in the FIP cohort ($P<0.0001$; Figure 1). Amongst pedigree cats, Persian (2.2% versus 9.2%), and Himalayan (1.1% versus 6%) were significantly under-represented (Figure 2). In contrast, several breeds were over-represented in the FIP positive cohort including British Shorthair (15.5% vs 5.7%), Devon Rex (8.9% versus 2.4%), and Abyssinian (4.4% versus 1.5%) cats.

Male cats were significantly over-represented in FIP positive cohort compared to the sex distribution of cats in Sydney ($p<0.001$; Figure 3). Entire cats were also significantly over-represented in the FIP positive cohort when compared to the Sydney cat population ($p<0.001$).[18] Cats under 2-years-of-age were significantly over-represented in the FIP positive cohort when compared to expected age

[1] All tables and figures are located at the end of the article before the references.

frequencies of the Sydney cat population ($p<0.0001$; Figure 4). Over the age of 2 years, all other age groups were significantly under-represented in the FIP positive cohort ($p<0.0001$).

DISCUSSION

This study compared the signalment of confirmed FIP cases with cats from the general cat population of NSW and Sydney. The results of this study lend further support to previous findings that age, breed, and sex predilections exist for FIP in Australia.[5,20-21]

Pedigree cats were significantly over-represented and domestic crossbreds under-represented in FIP cases when compared to NSW cat registration data, which is consistent with previous studies.[5,12-14] Certain pedigree breeds were significantly over-represented in the FIP cohort, especially the Devon Rex, British Shorthair, and Abyssinians while domestic crossbreds, Persian and Himalayan were underrepresented. There are similarities in the pattern of breed susceptibility to previously reported smaller case series in North America[14] and Australia[5] in terms of the overrepresentation of British Shorthair, Devon Rex, and Abyssinians and the under-representation of domestic crossbreds, Himalayan and Persian cats.

The finding that not all pedigree breeds were over-represented adds complexity to the notion that the key risk factor for pedigree cats is residing or beginning life in a multi-cat household. While this is certainly likely to be a contributing factor for such cats,[15] if coming from a multi-cat environment were the most important risk factor for FIP, one would expect all pedigree cats to be over-represented. As this is not the case and the over-representation of certain breeds may therefore indicate that particular breed lines within breeds are at increased risk of FIP. It has been suggested that there may be a genetic component to the efficacy of a cat's immune response and their subsequent susceptibility to FIP.[22-23] For example, individuals from certain breed lines are potentially at greater risk of inheriting susceptibility to FIP, particularly if they come from a small population with limited genetic polymorphism [22]. Genetic monomorphism at the major histocompatibility complex was implicated in a group of closely-related captive cheetahs devastated by an outbreak of FIP [22] resulting in the death of 60% of cheetahs. Direct relatives of cats that have died from FIP are significantly more likely to develop FIP than unrelated cats, suggesting that susceptibility to FIP is at least partly heritable.[23] The presence of susceptible lines within breeds could explain why not all pedigree cats are over-represented in FIP cases. It may also explain why the reported pattern of breed susceptibility differs between countries where different breed lines presumably exist.

Alternatively, individuals from certain breeds could be at increased risk of developing FIP because the catteries from which they originate may harbour more virulent strains of FIPV than other catteries[24] It is not within the scope of this study to determine whether the over-representation of certain breeds is due to the presence of susceptible bloodlines increasing the likelihood of *in vivo* mutation[23,25] or whether it is due to the presence of a more virulent virus within catteries of such breeds.[24] Nevertheless, identification of at-risk breeds or breed-lines may provide the basis for further study into potential genetic and epidemiologic factors and breeding practices that might play a role in FIP pathogenesis thereby facilitating the development of more effective preventative and treatment strategies.

Males were significantly over-represented in the FIP cohort. Sixty one percent of the FIP-positive cohort were male and this is higher than expected when compared to a previous report of population demographics, which found that males constituted 45% of the Sydney cat population[18] As with other feline diseases where sex predilections exist[26] behaviour (or co-morbidities linked with behaviour)

may be a contributory factor for males if they are indeed predisposed to FIP. Alternatively it could indicate a sex-linked component to a cat's immune response to FCoV.

The finding that entire cats were significantly over-represented in the FIP cohort is consistent with previous studies in which entire cats have been found to be over-represented.[13-14] In the FIP positive cohort however, entire cats were significantly younger than neutered cats, suggesting a confounding relationship between age and neuter status. This relationship is likely explained by the simple notion that many cats are neutered when they are over 6-months-of-age and thus younger cats are more likely to be entire. The over-abundance of entire cats in the pedigree population could relate to the notion that more of these cats are used for breeding than domestic crossbreds and that pedigree cats may be neutered later on average than domestic crossbreds. We therefore concluded that the significant over-representation of entire cats in FIP cases in the present study and the analyses of others were most likely due to confounding by age and purebred status.

The over-representation of young cats is consistent with other studies[5,12-15] although a bimodal age distribution for FIP cases was not evident in this study. There are several possibilities to explain the significant association between young age and FIP. Young cats have immature immune systems and are exposed to several major stressors such as weaning, desexing, vaccination, and re-homing, which may further compromise their immunity. They also have a higher prevalence of faeco-orally transmitted enteric pathogens (*Toxocara, Giardia, Tritrichomonas*), which may also contribute somehow to FECV replication.[27-28] These factors may eventually facilitate uncontrolled FIPV replication in macrophages and therefore lead to development of FIP in these cats.[6,29] The importance of the immune system in the pathogenesis of FIP was shown by Poland and colleagues (1996), who found that immune-compromised cats, such as those with feline immunodeficiency virus (FIV) infection, experienced higher FECV loads and were more likely to develop FIP than healthy FIV-negative siblings.[30] The over-representation of young cats in FIP positive cohort could also represent a temporal relationship between initial infection with FECV and subsequent development of FIP. The primary stage of FECV infection usually occurs in the first 18 months of life, a time frame closely mirroring the age group where FIP is most commonly diagnosed.[29] The clear predilection for FIP in young cats makes strategies aimed at minimising FECV infection in kittens, such as early weaning, seem a logical first step in preventing the development of FIP.[31] However, the ubiquitous nature of FECV necessitates advanced quarantine facilities and early prevention does not preclude development of FIP later in life, as evidenced by the wide age range observed in this study.

Registration data and recent census surveys were used as controls in this study in preference to FIP negatives on our FIP diagnostic test, due to known biases in sample submission and the reported low sensitivity of DIF. The use of these sample populations was not without fault however, as it could be argued that not all cats are registered and so registration data may also be subject to selection bias. Furthermore, the demographics of the Sydney and NSW cat populations may not be representative of the wider Australian cat population from which submission were drawn, and this may have limited the validity of our study. In order to conduct a truly accurate epidemiologic study, detailed data on the base population is clearly required. Unfortunately, no comprehensive census data about Australian cats is currently available. Determining such information may be critical to provide definitive answers regarding the epidemiology of FIP and other many other feline diseases in Australia.

The results of this study lend further support to previous findings that age, breed, and sex predilections exist for FIP. Future studies could continue to investigate why certain breeds appear to be predisposed to FIP while others are not, comparing apparently susceptible and more resistant breeds using the tools of contemporary genomics. Furthermore, the genomes of FCoV isolated from

catteries housing certain breeds experiencing a high incidence of FIP could be compared to viruses isolated from other catteries/breeds that have not experienced FIP, to determine whether differences between virus strains from different catteries account for the observed breed predilections for FIP. Development of a registry for FIP cases would enable tracking of FIP through breeds and breed lines, and this could help in the formation of controlled breeding plans should inherited susceptibility be confirmed as a true risk factor. In the meantime, veterinarians should continue to maintain a high index of suspicion for FIP in young cats and should be aware that FIP may be more likely in some breeds of cats than others.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare. This research received no specific grants from any funding agency in the public, commercial or not-for-profit sectors.

TABLES AND FIGURES

Table 1. Results of submissions to Veterinary Pathology Diagnostic Services for diagnosis of Feline Infectious Peritonitis by direct immunofluorescence or immunohistochemistry between 2004 and 2011.

	Number of submissions (%)		
Test	Positive result (case)	Negative result	Total
Direct immunofluorescenc	292	397	689 (80%)
Immunohistochemistry	90	89	179 (20%)
Total	382 (44%)	486 (56%)	868 (100%)

Table 2. Current cat registrations with the Companion Animal Register, NSW accumulated to the year 2011

Breed	Number of cats
Domestic crossbred	340,699
Burmese	20,929
Ragdoll	13,079
Siamese	7,531
Persian	6,906
Himalayan	5,869
British Shorthair	5,624
Birman	5,517
Russian Blue	4,129
Tonkinese	2,746
Other	26,116
Total	439,145

Figure 1. Observed domestic crossbred and pedigree cat frequencies in feline infectious peritonitis cases and expected breed frequencies based on registration data from the Companion Animals Register, NSW

***= Statistically significant difference between and observed and expected frequencies**

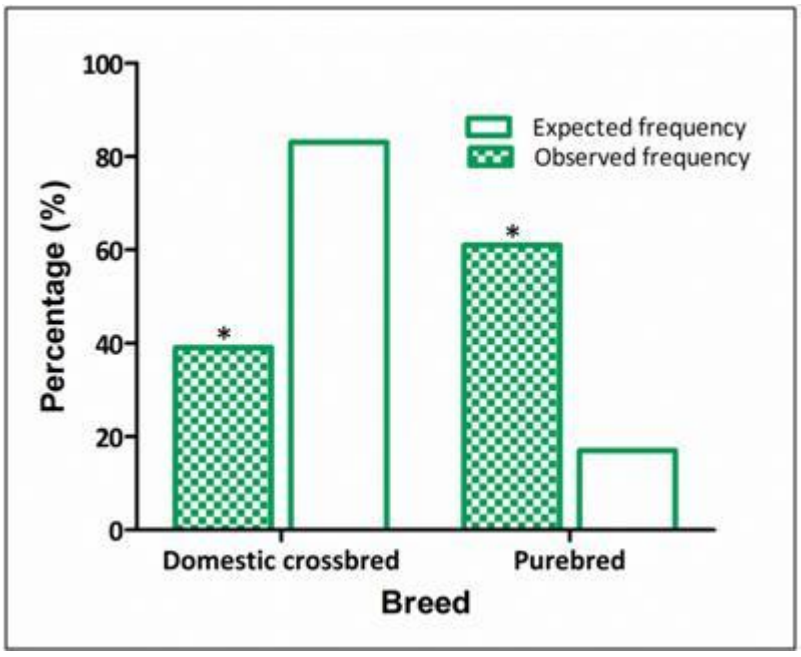


Figure 2. Observed frequency of each breed within pedigree cats with confirmed feline infectious peritonitis cohort and expected breed frequencies of pedigree cats based on registration data from the Companion Animals Register, NSW.

***= Statistically significant difference between and observed and expected frequencies**

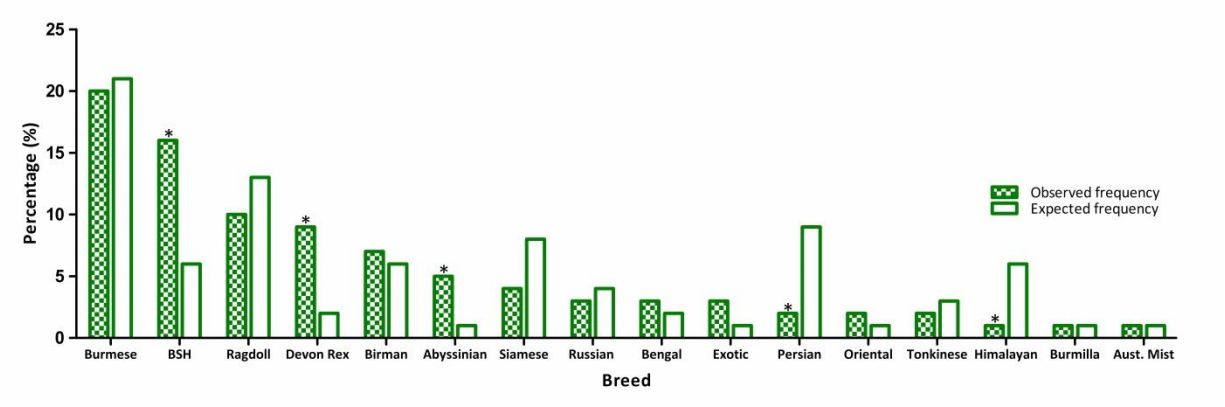


Figure 3. Observed sex frequencies in feline infectious peritonitis cases and expected sex frequencies based on a demographic study into cats in Sydney, NSW. (Toribio *et al*., 2009)

***= Statistically significant difference between observed and expected frequencies ($p<0.05$)**

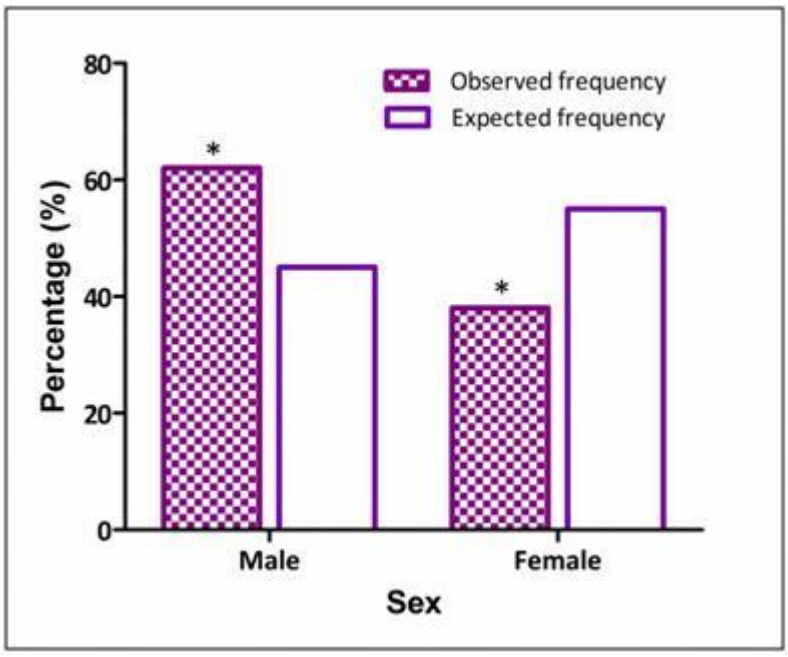
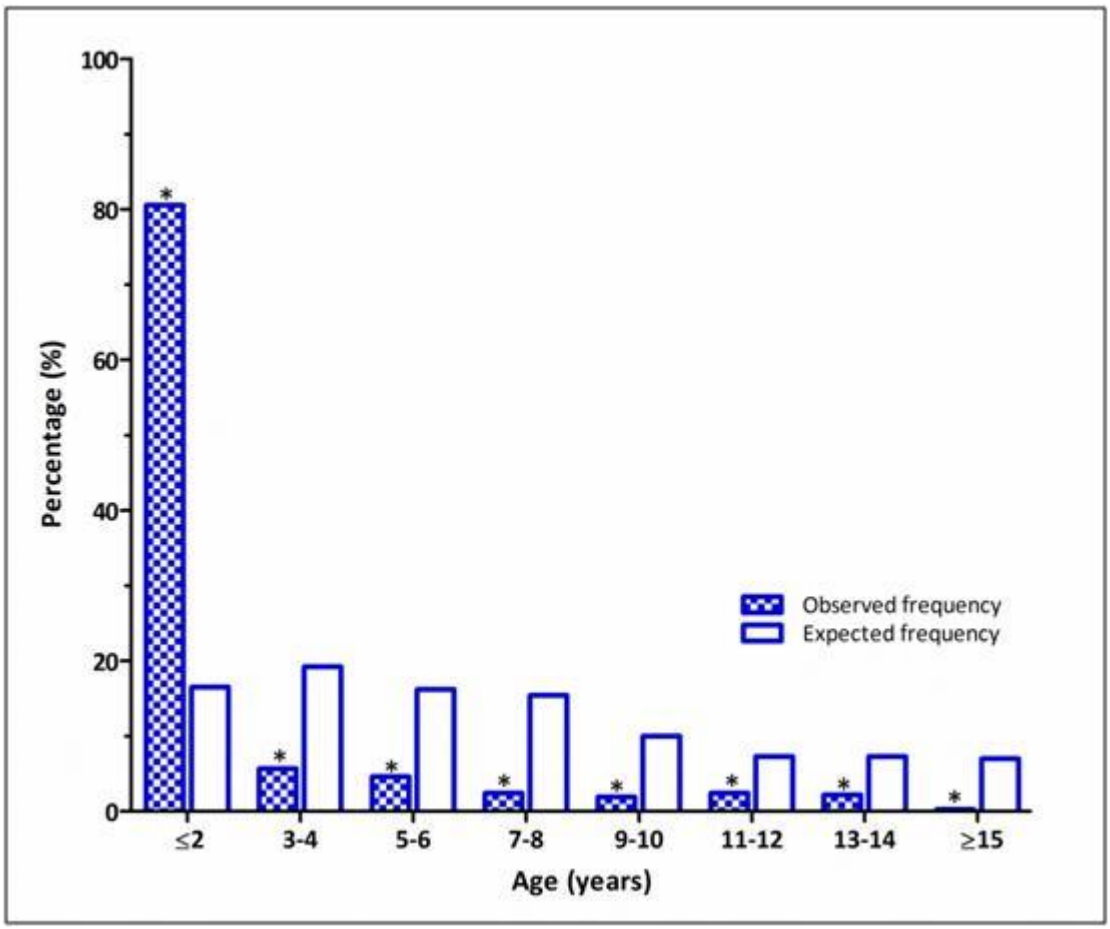


Figure 4. Observed age frequencies in feline infectious peritonitis cases and expected age frequencies based on a demographic study into cats in Sydney, NSW. (Toribio *et al*., 2009)

***= Statistically significant difference between observed and expected frequencies ($p<0.05$)**



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