Risk of feline infectious peritonitis in cats naturally infected with feline coronavirus

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Summary

A longitudinal survey of 820 cats in 73 households was conducted over a period of 6 years to establish the fate of pet cats that were seropositive after natural exposure to feline coronavirus (FCoV). In particular, their risk of developing feline infectious peritonitis (FIP) was determined. The seropositive cats were assigned to 1 of 3 groups: cats from households in which FIP had recently been diagnosed; cats from households in which FIP had not been diagnosed, but from which kittens had been relocated and subsequently died of FIP; and cats from households in which FIP had not been diagnosed. Cats in the first group were not at greater risk of developing FIP than were cats in the other 2 groups. Consequently, any household in which seropositive cats live must be considered a potential source of FCoV that can cause FIP. There was no evidence that the enhanced disease, which has been described after experimentally induced infection of seropositive cats, exists in nature. Thus, analysis of the survival of the seropositive cats over periods of up to 36 months indicated that their risk of developing FIP decreased with time, suggesting the development of immunity rather than increased susceptibility to disease. In addition, of 56 cats deemed to have been naturally reinfected because their anti-FCoV antibody titers decreased and subsequently increased, only 3 developed FIP.

Essentially, all cats exposed either naturally or experimentally to feline coronavirus (FCoV) develop FCoV antibodies.[1] In the absence of a routinely used test for the detection of the virus itself, serologic testing has been the only aid to the diagnosis of FCoV infection and feline infectious peritonitis (FIP). The diagnostic potential of serologic testing has been limited because the presence of antibodies neither indicates that a cat is a shedder of virus nor that it has FIP. However, to the authors' knowledge, the risk of subsequent development of FIP in seropositive cats has not been rigorously defined. The primary objective of the study reported here was to assess this risk. A survey was conducted in which 820 cats in 73 households in the United Kingdom that had endemic FCoV infection were monitored for periods of up to 6 years to determine the outcome of their exposure to virus. Wherever possible, samples were obtained for histologic examination and confirmation of clinical diagnosis from cats that developed disease.

In addition, the study afforded the opportunity to examine whether certain features of FCoV infection by laboratory-adapted strains of virus also developed after natural infection of pet cats. Two particular features were investigated. Because laboratory strains of FCoV may cause either FIP or enteric disease,[2] it was considered important to determine whether FIP was more likely to develop in seropositive cats that had been in contact with cases of FIP, and, therefore, such cats might be supposed to have encountered a virulent form of virus, than in cats that had been exposed to other seropositive cats that had enteric disease or were healthy. Also, an enhanced form of FIP has been described in seropositive cats that were subsequently challenge exposed with strains of FIP-causing FCoV.[3] Enhanced FIP manifests itself in various ways: either a higher proportion of cats develops FIP than does that in the control group of previously unexposed cats, or the course of the disease is accelerated (< 2 weeks compared with > 1 month), or cats with enhanced disease may have gross pathologic features atypical of FIP. We investigated whether enhanced disease also developed in a field setting. The information gained from this study will be useful to veterinarians in advising clients on the risks to seropositive cats in particular home environments.

Materials and Methods

Diagnosis of FIP—Clinical diagnosis of FIP was confirmed by histologic examination wherever possible. Presumptive diagnosis of feline enteric coronavirus (FECV) infection was made according to a clinical history of nonresponsive diarrhea principally in kittens 5 to 12 weeks old,[1,2,4,5] or, in 1 household,

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because histologic examination of specimens from a kitten revealed stunting and fusion of the small intestinal villi similar to that caused by transmissible gastroenteritis virus infection in young pigs.

Histologic examination—Tissue specimens were fixed in neutral-buffered 10% formalin and embedded in polywax before sectioning. Sections were stained with H&E. Diagnosis of FIP was made when there were characteristic lesions of vasculitis with a central area of necrosis surrounded by a perivascular infiltration of mononuclear cells, proliferating macrophages and lymphocytes, plasma cells, and neutrophils.[6-10]

Statistical analysis—Life tables were constructed to illustrate FIP-specific mortality over time, using the BMDP program 1L.[a] Death from causes other than FIP or cats that were relocated were treated as censorings (ie, they were treated as if they had been lost to follow-up at that point). This survival analysis was performed for the entire population, and was repeated for the 3 individual groups. The survival experience in the 3 groups was compared, using the log-rank test.

Serologic testing—Antibody titers were determined by use of indirect immunofluorescence.[11]

Diagnosis of FeLV and feline immunodeficiency virus (FIV) infections—The adult cats in 43 households were screened for FeLV and, in 29 of these households, were also tested for FIV. Samples of plasma were screened for FeLV antigen, using an ELISA[b] to detect p27 antigen, and positive results were confirmed by virus isolation.[12] Antibodies to FIV were detected by use of another ELISA.[c]

Results

A follow-up study of cats naturally exposed to FCoV was begun in 1988 to monitor the antibody status of FCoV-seropositive cats and the seronegative cats with which they were in contact. Seventy-three cat owners participated, and 820 cats were monitored for variable periods. The fate of kittens born into these households has been described in detail elsewhere.[11,13]

At the beginning of the survey, antibody testing for FCoV was carried out monthly. However, little change was found in antibody titer from month to month, so testing thereafter was at intervals of 3 to 6 months or whenever owners would permit it. Details of the health of each cat, whether it was free-ranging or kept indoors, and the number and antibody titer of its contacts were recorded on a database designed for the survey.

Reasons for household participation were determined (Table 1). Group-A cats were initially tested for antibodies to FCoV because a cat in the same household had died of FIP. Thus, it was presumed that virulent FCoV was, or had been, present in group-A households. Group B consisted of households from which a kitten was sold and subsequently developed FIP in its new home. Hence, it was likely, but not certain, that cats in these households also had been exposed to virulent virus. Group C comprised households with cats that had been serologically tested for a variety of reasons, but had no history of FIP at the time of joining the survey. Cats of group-C households were presumed to be exposed to either enteric or avirulent coronaviruses.

Table 1—Reasons for participation of households in the survey

Group	Reason for participation	No. of households	Households with subsequent FIP
A	Cat in household died of FIP	33	9
B	Relocated kitten died of FIP	14	5
C	No history of FIP in the household	26	7
	FCoV was in the differential diagnosis	4	2
	FECV infection was suspected	3	2
	Contact with suspected FCoV excretor	8	1
	Routine test prior to mating	7	1
	Unknown	4	1
Total		73	21

FIP = feline infectious peritonitis; FCoV = feline coronavirus; and FECV = feline enteric coronavirus.

Household size ranged from 1 to 42 cats. Few households remained constant in size during the entire period of the survey because cats died and new cats were introduced. Ninety-seven cats were lost to the survey because they were relocated.

The risk of developing FIP decreased with time of observation of seropositive cats. A survival curve for the entire survey population of 820 cats in which the proportion of cats surviving was plotted against months from the time a cat joined the survey (Fig 1). The curve was truncated at 36 months because there were too few deaths attributed to FIP ($n = 6$) among the remaining 188 cats to permit statistical analysis. It can be seen that the curve decreases steeply during the first few months and levels off by 6 months. Mean ± SEM FIP-specific mortality was 4.8 ± 0.9% at 36 months.

Feline infectious peritonitis developed in cats from households even when there was no previous history of FIP. Survival curves were plotted (Fig 2) for each group of cats according to the tabulated groupings (Table 1). During the course of the survey, group A contained 420 cats, group B contained 110 cats, and group C contained 290 cats. Feline infectious peritonitis developed in group-C household cats in which

[a] IL Program, BMDP Statistical Software Inc, Los Angeles, Calif.
[b] FeLV Petcheck, IDEXX Labs Inc, Westbrook Me.
[c] FIV Petcheck, IDEXX Labs Inc, Westbrook, Me.

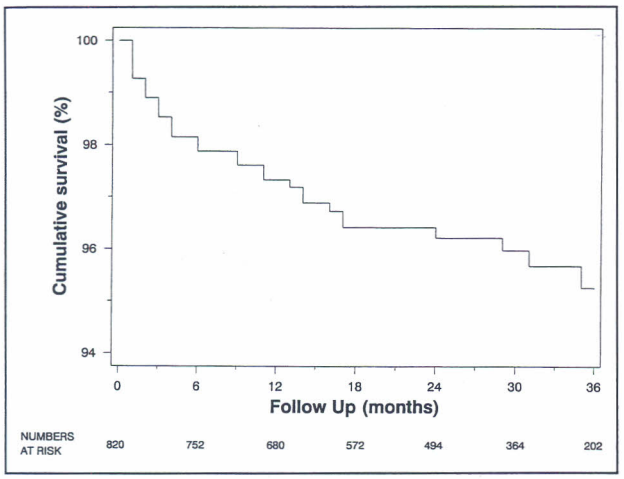

Figure 1—Survival curve of all the survey cats indicating that the probability of dying of feline infectious peritonitis (FIP) was greatest during the first 6 months into the survey. At 36 months, the probability of not developing FIP was 95.2%.

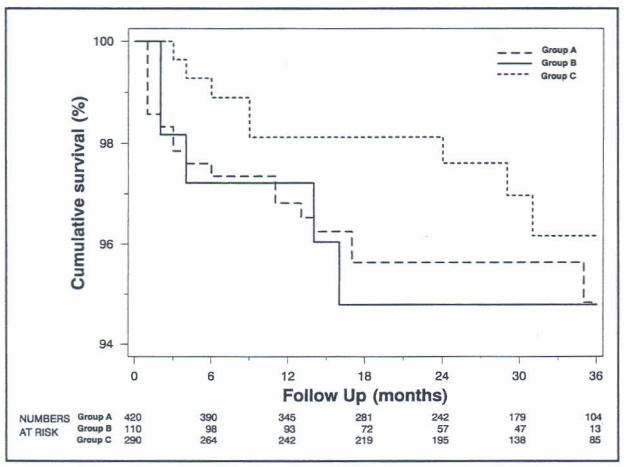

Figure 2—Survival curves of cats in groups A, B, and C indicating that the survival curves for all 3 groups were similar.

the clinical history suggested infection with an enteric or avirulent coronavirus, as well as in household cats of groups A and B, which appeared to have virus capable of causing FIP. Although cats in group C may have appeared to have higher survival rate than cats in the other groups, there was no statistically significant difference between the 3 survival curves (log-rank $\chi^2 = 1.45$, 2 *df*, $P = 0.48$).

Not including the index cases, 20 cats in group A developed FIP; 17 died during the first 17 months and the remaining 3 died at 35, 46, and 56 months. Thirty-nine cats died of other causes. Six group-B cats developed FIP (5 within 16 months and the sixth at 38 months), and 11 died of other causes. Eleven group-C cats developed FIP (5 within the first 9 months and the others at 24, 29, 31, 38, 48, and 65 months), and 37 cats died of other causes.

The incidence of FIP in kittens born into households in each group was determined (Table 2). The disease developed in 5% of seropositive kittens in group A, 9% of seropositive kittens in group B, and 9.6% of seropositive kittens in group C. Thus, kittens raised in a household with seropositive cats but no history of FIP are just as likely to develop FIP as those born into households with previous history of FIP.

Table 2—Incidence of FIP in kittens born in survey households

Group	Breeding households	Seronegative kittens	Seropositive kittens	No. that died of FIP*
A	17	86	102	5 (5%)
B	14	49	66	6 (9%)
C	14	152	114	11† (9.6%)

* Values in parenthesis are percentage of seropositive kittens that developed FIP. No seronegative kitten developed FIP. † Seven of these kittens were from 1 household in which 48 kittens were seropositive and 21 kittens were seronegative.

There was no evidence of enhanced disease when cats were re-exposed naturally to FCoV. One feature of enhanced disease is that more cats die when exposed to virulent FCoV if they have previously encountered FCoV. If cats previously exposed to FCoV were more likely to develop FIP than FCoV-naive cats, the incidence of FIP (and, therefore, the steepness of the steps in the survival curve) would be expected to increase with time. From the results (Fig 1), this situation obviously did not develop. In fact, the longer a cat survived after exposure to FCoV, the lower was its risk of developing FIP, suggesting that the cats developed some degree of resistance to FIP.

The survival curves were designed to examine the survival of known FCoV-seropositive cats. However, more than half the seropositive cats in 24 of the 73 households became seronegative, suggesting that the infection died out in these households. Therefore, the cats in a high proportion of the households that were seronegative may not have been susceptible to induction of enhanced disease. Consequently, we compared mortality at the time of development of subsequent cases of FIP with mortality at the time when the index case was recognized. If cats that had been exposed previously to FCoV were sensitized, the mortality might be expected to be greater at the time subsequent deaths attributable to FIP occurred than on the occasion of initial case. Group-A households joined the survey because a cat had died of FIP. Mortality at the time of the index case was calculated by dividing the number of cats that died of FIP within 3 months of development of the index case, including the index case (n = 43), by the total number of group-A cats available to develop FIP at that time (n = 304), and was found to be 14%. There were further cases of FIP in adult cats in 9 of the households in group A. Fourteen cats died and 158 in-contact cats survived, yielding a mortality of 8.8%, which is significantly less than mortality at the time of the first recorded death ($\chi^2 = 3.8$, $P < 0.05$). Not counting the initial FIP cases in group A, mortality in cats of all groups was 7% (24 cats with FIP, 341 cats in contact), which is significantly different from initial mortality in group-A cats ($\chi^2 = 8.6$, $P < 0.005$). Thus,

the FIP mortality was less at the time of second or third deaths attributable to FIP than at the time of the initial death, again suggesting development of resistance, not susceptibility.

Cats in other households were re-exposed to FCoV, as was evident from the decrease then increase in antibody titer. However, no cat in these households died of FIP, so it was impossible to determine whether the cats had been infected with a virulent form of the virus. Therefore, these cats were not included in the analysis.

The question of enhanced disease was also examined by observing the fate of individual cats. Fifty-six cats were observed in which antibody titer decreased at least threefold, then increased again, indicating reinfection. Although there was no way of assessing virulence of the FCoV to which these cats were re-exposed, they were from 21 households, so it was likely that they were infected with a range of FCoV strains and doses. Cats in which antibody titer decreased to zero, then increased, were not included because the intention was to examine cats that had been re-exposed to FCoV when they were seropositive.

Only 3 of these 56 cats (No. 5047, 5340, and 5611) succumbed to FIP. Cats 5340 and 5611 were tested at the time of increase in antibody titer because they had clinical signs of FIP (ascites in cat 5611, and weight loss and lethargy in cat 5340). Cat 5340 died of effusive FIP 10 weeks after its antibody titer increased. Cat 5611 was already manifesting signs of effusive FIP at the time its increasing antibody titer was detected, and it died 12 days later. The antibody titer of cat 5047 increased to 1,280 in November 1990, and the cat died in June 1991. Although it was impossible to tell exactly when these cats were subjected to reinfection, none died with hallmark signs of enhanced disease. Thus, cats did not die more rapidly nor did the disease seem to be more severe than that attributable to conventional FIP.

Of the 56 cats, 5 died of non-FIP related conditions, and histologic examination of all major organs was done for only 2 cats; histologic examination of a mammary tumor specimen was done for a third cat. There was no evidence of FIP lesions in these cats. Death in these 5 cats was attributable to: death during parturition; euthanasia because of chronic diarrhea and concurrent FeLV and FIV infection; mammary adenocarcinomata; chronic interstitial nephritis; and accidental death. These cats died 14, 71, 95, 97, and 127 weeks after their antibody titer increased. Thus, we did not miss cases of enhanced FIP in these cats because of atypical clinical signs of disease.

The mean time from antibody titer beginning to decrease then increasing again, indicating reinfection, was 70.4 weeks (range, 25 to 167 weeks).

Discussion

Although FIP was first described over 30 years ago,[14] as far as we are aware, this study is the first record of a longitudinal survey of the fate of pet cats naturally infected with FCoV. There were 3 major conclusions from the study, some of which were surprisingly different from those drawn from results of experimentally induced infections. Cats introduced or born into households with no history of FIP were as likely to develop FIP as were cats or kittens in households with a history of FIP. Also, the risk of an individual cat developing FIP decreased with time, implying that immunity rather than susceptibility developed. In addition, natural reinfection of FCoV-seropositive cats did not lead to enhanced disease.

Cats were most likely to develop FIP within the first 6 months of joining the study, no matter to which household group they belonged. The risk of any individual cat dying of FIP was only 4.8% at 36 months into the survey, and FIP-caused death was infrequent thereafter. Selecting only households that were presumed to have virulent virus exposure because a cat developed FIP, mortality was 14% at the time of the first death attributable to FIP and was 7 to 8.8% at the time of subsequent development of cases of FIP. The most likely explanation for this difference is that at the time of the initial death, many of the cats had encountered FCoV for the first time and, therefore, had no immunity; by the time they were reinfected, some cats had developed immunity.

Two possible arguments against this explanation are that later deaths may have been a result of FCoV infection acquired at the time of the initial FIP case in the household, or that the cats may have subsequently been infected with a less virulent virus. It might be supposed that later deaths attributable to FIP were simply the result of the notoriously long incubation period of the disease and that the virus had disappeared from the households by the time of later deaths, so that the cats which developed FIP subsequently were not, in fact, re-exposed to the virus. This was not the case, however, because later deaths often occurred in newly introduced cats or kittens born into the household, indicating that active infection persisted in these households. The second alternative explanation for the decrease in mortality is that the cats subsequently were exposed to a less virulent virus. However, some cats and kittens developed FIP, so viruses capable of causing FIP must have been present. Deaths in each household were sporadic, occurring singly or in pairs, so that, although virulent virus was present, either it did not spread or some factor(s) other than virulence determined whether cats developed FIP.

In the survey, FIP developed as frequently in households that had no previous history of FIP as in those that had such history. Several explanations could be offered for this finding. It is possible that virulent viruses were introduced into group-C households after the households joined the survey. If this were the case, one would have expected that the survival curve for group-C households would be shifted to the right, compared with the curves for groups A and B because in group-C cats, the disease would be expected to take longer to develop. Although the group-C curve appeared to be shifted to the right, the difference was not statistically significant. Also, cats may initially have been infected with several FCoV of variable virulence.

Still another possibility is that development of FIP may be related to the infective dose of virus or the state of the cat's health and immunity at the time. However, others have observed that FIP can arise in cats infected with FECV, from which it has been proposed that FIP may arise by mutation or recombination in FECV, which allows the variant virus to grow in macrophages in addition to intestinal epithelial cells and, thereby, to invade the body systemically.[4,15,16,d]

Whichever explanation is correct, the practical consequence in the field setting is that precautionary measures should always be taken to prevent spread of FCoV infection to susceptible cats and kittens,[11,13] whether or not a history of FIP exists in the household. Testing of cats for FCoV antibodies has been criticized because distinction could not be made on the basis of serologic testing between laboratory isolates of FCoV of variable pathogenicity.[17] It was argued that if the coronavirus infecting a seropositive cat were avirulent or enteric, the finding of antibodies was irrelevant and the cat could be safely used for breeding or moved into an uninfected household. Because the mortality attributable to FIP is low, cat owners with small numbers of cats may wait for a considerable period before they lose a cat to FIP and, consequently, the lack of a clinical case of FIP might cause veterinarians to conclude that their clients' cats were infected with an avirulent or enteric coronavirus which did not have the potential to cause FIP. Our results indicate that this is an unwise assumption.

Another apparent difference between the situation in the laboratory and in the field setting concerned occurrence of enhanced disease. Enhanced disease has been observed particularly in vaccine trials, in which vaccinated, and therefore seropositive, cats were more likely than unvaccinated cats to develop FIP after challenge exposure.[3] In our study, we found that re-exposure of cats in the field did not induce enhanced disease. A likely reason for this difference is that the dose of virus to which cats are exposed may be greater in experimentally induced infections than in natural infections. Experimentally, sublethal amounts of virulent virus were documented to be protective, but high doses were almost always lethal.[18] Alternatively, the difference may be a consequence of the route of transmission used in experimentally induced infections, in which virus is often administered parenterally, bypassing the mucosae, which are the first line of defense against natural FCoV infection. Parenteral inoculation is often necessary for transmission because it may be difficult to infect cats orally with FCoV.[8,19] For example, in 1 experimental attempt to infect cats orally, up to 6 virus exposures were administered, but finally, SC inoculation was required to induce seroconversion and disease.[19]

Analysis of the entire survey population for enhancement meant that cats were inevitably included whose antibody titer had decreased to zero by the time they were reinfected. To examine specifically whether currently seropositive (as opposed to previously infected) cats were more likely to develop enhanced FIP, 56 cats were observed whose antibody titer decreased then increased again, indicating reinfection. No doubt, other cats with more even antibody titer throughout the survey also were re-exposed to virus, but there was no way of detecting these cats. Only 3 of the 56 cats died of FIP, and the time course in these cats was not accelerated nor were the pathologic findings bizarre. We believe that these cats were reinfected rather than that they had a reactivated latent infection, because in households in which over half the cats became FCoV-seronegative then seroconverted again, the source of virus could usually be traced to contact with an infected cat from outside the household, suggesting that the source of reinfection had not been recrudescence of virus excretion by the remaining seropositive cats.

These findings help to answer the practicing veterinarian's question of whether it is safe to introduce a seropositive cat from one household into another household of seropositive cats. Previously, a concern was that exposure to another strain of FCoV would result in increased susceptibility of either the new cat or the incumbents to FIP. We now know that this outcome is unlikely.

In conclusion, cats naturally infected with FCoV appear to be immune and not to develop enhanced disease on reinfection. Conclusions cannot be made about the virulence of the FCoV infecting a household of cats on the basis of clinical history, and all FCoV infections should be treated as potentially able to lead to development of FIP.

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