

An Update on Feline Infectious Peritonitis

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Introduction

Feline infectious peritonitis (FIP) is an important disease to the cat clinician for several reasons; it is fatal in most (clinical) cases, its biology is poorly understood and prevention is difficult, to say the least. It is also an enigmatic disease: a sporadic viral condition is a contradiction in terms. One expects epidemic expansion from virus diseases, or at least a consistent pattern of spread in a cat society. Another mystifying trait is the fact that antibodies - the molecules we associate with immunity and protection - have no beneficial effect for the cat. Indeed, under certain circumstances they may even precipitate disease, causing the 'early death' phenomenon. The detection of antibodies is of no benefit to the consulting veterinarian either, since titres are meaningless for the purposes of diagnosis and prognosis in individual patients. Nevertheless, there is a place for serology, as we will discuss later. There is also a vaccine available in some countries, which has been shown to provide some protection [4]. However, its efficacy is a matter of debate.



Fig. 1 A pronounced case of the exudative or 'wet' form of Feline Infectious Peritonitis (FIP). Clearly, the cat is emaciated with a pronounced extension of the abdomen.

Feline coronaviral polyserositis, as it should be termed, is the fatal 'tip of the iceberg' to a common feline infection with a group of ubiquitous viruses. Most of these coronaviruses are harmless and perfectly adapted to growth in the gut. They have been named 'feline enteric coronaviruses', to distinguish them from the killer viruses that replicate in the feline macrophage. Persistently infected, healthy cats play the most important epidemiologic role in FIP, because by harbouring feline coronaviruses (FCoVs) in their intestines and blood, they act as a constant source of infection. The virus is shed in the faeces, saliva and perhaps other body fluids of infected cats. In addition to these 'pathotypes', coronaviruses also occur in two serotypes, both of which can cause FIP after having undergone subtle genetic changes. It is only for reasons of convenience that we shall continue to

use the term FIP virus (FIPV) - to denominate those FCoV strains that carry the mutation(s) responsible for the increase in virulence. The legitimacy of such a nomenclature is questionable, however; it is like giving different names to a virus and its attenuated vaccine strain.

A virologist's perspective

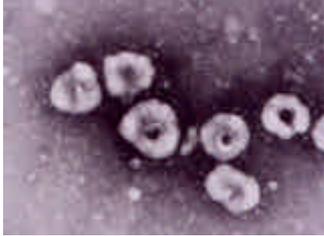


Fig. 2 Electron micrograph of coronavirus particles. The name of this RNA virus family is derived from the name of the halo seen around the sun during an eclipse, the 'corona solis'. It refers to the fringe of petal-shaped spikes ('peplomers') radiating from the viron membrane. The peplomers carry epitopes, which induce neutralizing antibodies and provide protection against coronaviral disease, for example in pigs (transmissible gastroenteritis) and chickens (infectious bronchitis), but are at the base of the 'early death' phenomenon in cats.

Coronaviruses (genus *Coronavirus*, order *Nidovirales*) are common pathogens found in mammals (causing a form of 'common cold' in man, transmissible gastroenteritis in swine, diarrhoea in cattle and other conditions) and birds (giving rise to infectious bronchitis in chickens and bluecomb disease in turkeys). They are enveloped viruses, with an RNA genome about 30 kilobase in length, making theirs the largest of all RNA genomes. It is generally accepted that one out of 10,000 nucleotides is changed in any round of RNA genome replication. Consequently, myriads of copying errors can be expected: since the coronaviral genome holds about 30,000 nucleotides, one would differ from the next at least at one site. Thus, no two coronavirus particles are genomically identical - a notion that has led to the so-called 'quasispecies' concept.

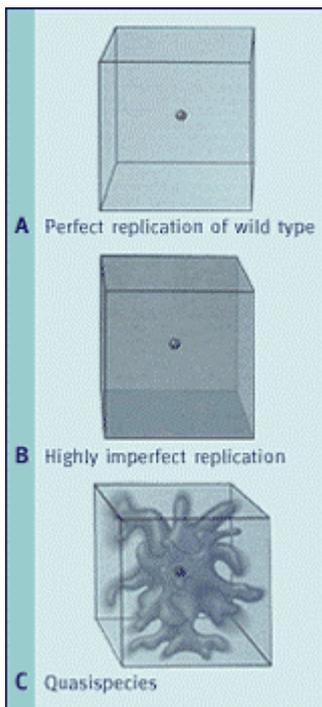


Fig. 3 The quasispecies concept in viruses. The 'sequence space', indicated by the cube, represents the theoretically possible nucleotide variations in a replicating genome. A faithfully replicating genome - like that of a vertebrate - would occupy a point-like niche in sequence space, a hypothetical viral genome with unlimited degrees of freedom (all variants allowed and infectious) would fill the entire sequence space. Due to their poor replication fidelity, with 1:10,000 to 1:100,000 base substitutions per site, RNA viruses occupy a cloud-like niche in sequence space. This is the stuff of viral evolution: during transmission the virus goes through a population bottleneck and expands to a new 'quasispecies cloud' in the new host.

Viruses evolve more than a million times faster than cellular microorganisms, and one wonders how they can maintain their identities as pathogens over any evolutionarily significant period of time. As the Nobel laureate Manfred Eigen [3] exclaimed: "Why didn't they mutate out of existence?" The answer to this question is, of course, that individual viruses do not count biologically but rather a cloud of variants expanding around a 'consensus' sequence.

Although generally associated with acute, self-limiting enteric and respiratory infections, coronaviruses can also establish persistent infections. In vivo these have mostly been studied using mouse hepatitis virus as a model; suckling rodents

may develop a chronic demyelinating disease not unlike multiple sclerosis in man, with viral replication in the central nervous system. From such animals, virus was isolated as late as one year after inoculation. Only a few studies have addressed the role of viral persistence during natural coronavirus infection, and FIP is now the most prominent example.

Feline coronaviruses cause mild enteric infections in almost all catteries in Western Europe and America (for a review see [5]). The low-virulence 'enteric' FCoV and the disease-causing FIPVs are genetically closely related [6], and we think that the latter are virulence variants of the former, which arise in individual FCoV-infected hosts [12,15]. This means that no two cases of FIP are caused by identical viruses and that horizontal transmission, that is cat-to-cat transfer, is the exception rather than the rule.

On the basis of in vitro neutralization tests FCoVs can be allocated to one of the two serotypes mentioned above. Type I is prevalent in Europe, is found in most fatal cases of FIP, but is the least studied because of its reluctance to grow in culture. The type II FCoVs are more common in other parts of the world (e.g. Japan) and are a showcase of viral evolution. They arise from RNA recombination events during which genetic information from the canine coronavirus is incorporated into FCoV type I genomes [9,15].

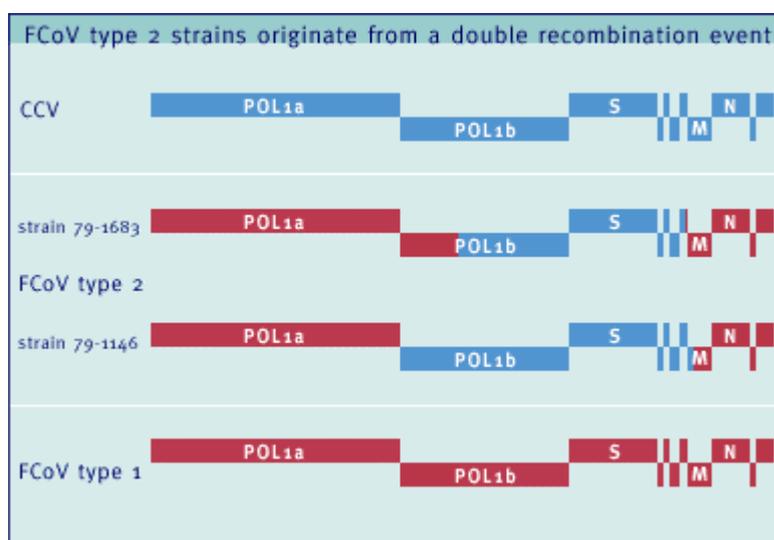


Fig. 4 Genomic organization of and recombination between carnivore coronaviruses. The boxes represent the genes responsible for the 'structural' proteins building the virus particle, such as the 'peplomers' (see legend to Fig. 2) or spikes (S), two membrane proteins (E and M) and the nucleocapsid protein (N), which wraps the genome. POL stands for polymerase, and also the genes 3 and 7 code for non-structural proteins; mutations in the 3c gene have been found in coronavirus infected cats that developed FIP. The uppermost graph symbolizes the genome of a canine coronavirus, the lowest that of a feline coronavirus. Recombinants between both have been found in the field, and the varying cross-over sites indicate that this event occurs regularly.

The FCoV carrier state

Epidemiological studies suggest that FCoVs may cause persistent infections, that a carrier state exists, and that many infections are not cleared by the cat's immune system - or only after a long time. It is common knowledge that healthy cats with coronavirus antibodies may cause seroconversion in contact animals within 2 to 10 weeks. The infection is spread presumably via the fecal-oral route, and some of the contact animals will subsequently succumb to FIP [1]. First solid evidence for a carrier state came from an experiment in which cats were infected with a sublethal dose of tissue culture grown FIPV and kept in isolation. To induce FIP, the cats were superinfected with feline leukaemia virus, which is known to be strongly T-cell immunosuppressive. From this work it appeared that FIPV could persist in the experimentally infected host for at least 4 months [11].

To identify asymptomatic FCoV carriers and to monitor virus shedding, a group of scientists from Utrecht developed a nested RT-PCR assay targeted to a highly conserved (invariant) region of the FCoV genome. Using this assay, viral RNA had previously been detected in the faeces, tissues and body fluids of cats with FIP [2,9]. Interestingly,

however, FCoV RNA was also found in the faeces, and occasionally in the serum, of perfectly healthy cats. The virus persistence and evolution was then studied in a closed cat breeding facility in Hanover/Germany with an endemic FCoV type I infection. Viral RNA was detected in the faeces and/or plasma of 85% of the cats tested. Of five cats identified as FCoV shedders during the initial survey, four had viral RNA in their faeces when tested almost four months later. This could be due either to repeated reinfections or to persistence of the virus in the cat's organism. To distinguish between these possibilities, two cats were placed in perfect isolation, and fecal virus shedding was monitored every 2-4 days. In one cat, shedding continued for up to seven months. The other animal was sacrificed after three months of continuous shedding since we wanted to find the sites of viral replication. Viral genomic RNA was found in almost any tissue tested, but messenger RNA (which is synthesized only when a virus multiplies) was detected exclusively in the ileum, colon and rectum; in these parts of the gut individual FCoV-infected cells (i.e. not large parts of infected tissue) were also spotted by immunohistochemistry. These findings provide first formal evidence that FCoV causes chronic infections [10].

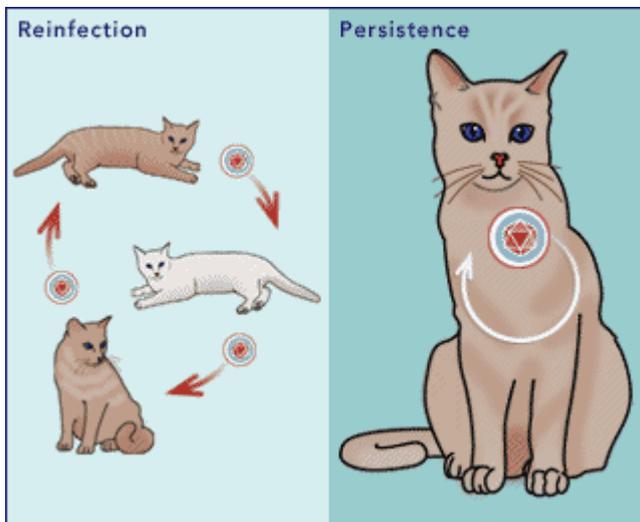


Fig. 5 The difference between recurrent and persistent infections. Viruses may be maintained in a population either by animal-to-animal transfer or by prolonged presence in an individual's body. As we learn more about viruses, the persistent infection appears to be the rule rather than the exception - and it occurs also in feline coronaviruses.

Recently, a modification of the conventional RT-PCR procedure was introduced - the so-called TaqMan technique. It allows testing of many samples within a short period of time, and in addition permits the reliable

quantitation of FCoV genomes in a sample, e.g. in rectal swabs taken from individual cats [7] (for details of the TaqMan technique, look into the Tools section of this VetSciTe issue). Using this technique, the coronavirus shedding pattern was determined over a period of 24 weeks in 77 cats kept in multi-cat household situations. We found a highly significant correlation between the amount of FCoV shed in faeces and the frequency of shedding [13]

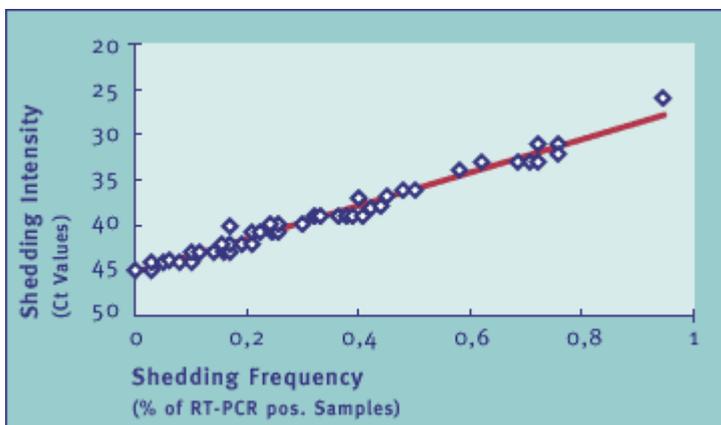


Fig. 6 Correlation between shedding frequency and shedding intensity in 77 cats followed for 24 weeks. It becomes clear that with higher shedding frequency the amount shed is significantly increased ($r=0.9895$, $p<0.0001$). The amount of FCoV shed in 1 g faeces with a Ct value of 30 equals $10 \exp. 7$ viral particles. The shedding frequency in these cats was found to be low (<30% of all samples positive) in 78%, intermediate (between 30% and 90% of all samples positive) in 21% and high (>90% of all samples positive) in 1% of the cats.

To study viral evolution during chronic infection, the FCoV's sampled from individual cats were characterized. Phylogenetic comparisons of sequences obtained for independent European and American isolates indicated that the viruses in the breeding facility form a clade (a closely related cluster) and are likely to have originated from a single founder infection. Each cat harboured distinct FCoV quasispecies with immune selection (antigenic drift) occurring during chronic infection.

These data support a model in which chronic carriers maintain the endemic infections in cat societies. Virtually every kitten born in a breeding facility becomes infected, probably from its queen[1], as soon as its maternal protection wanes. Once infected, the cats appear to resist superinfection by closely related FCoVs, every cat carrying its private, harmless clan of variants.

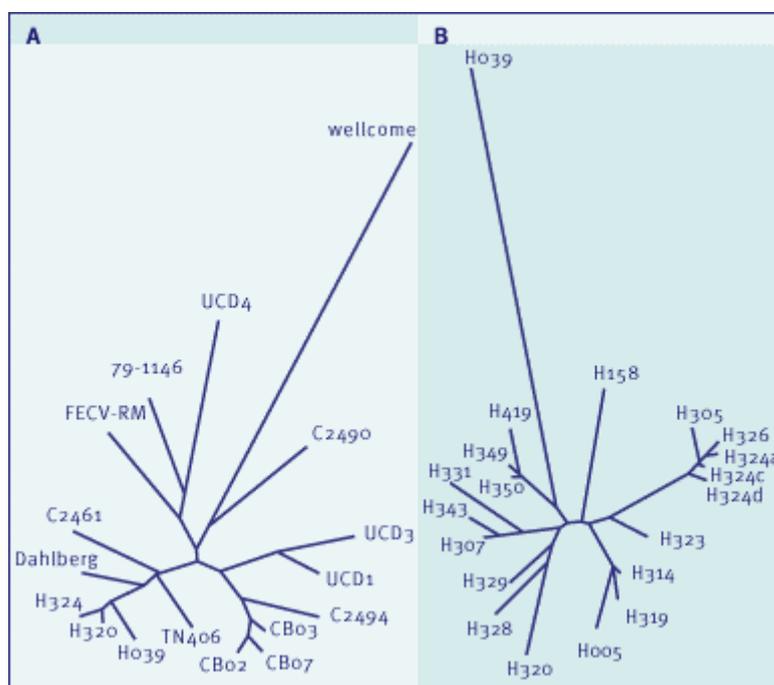


Fig.7 Unrooted 'phylogenetic trees' showing genetic relationships between feline coronaviruses, where branch length indicates evolutionary distance. Graph A illustrates the relationship between coronaviruses shed by cats in a closed breeding facility in Hanover, Germany (isolates indicated by H) as compared with laboratory strains; field strains from the Netherlands (C, Dahlberg) and the United States (RM) are included. The non-structural gene 7b was used in this comparison. Panel B shows a more detailed analysis - of the structural S gene - with in the Hanover cattery alone. Clusters of more closely related viruses can be distinguished, most of them from littermates. Amino acid substitutions were not random but are linked to predicted epitopes, indicating antigenic drift.

At the 1997 WSAVA/BSAVA Congress in Birmingham, workers from Bristol University first presented data that confirmed this epidemiologic concept using a different approach. These workers were indeed able to culture FCoVs from the blood of healthy cats from seropositive catteries. Blood samples obtained from healthy cats of nine different breeds from nine separate catteries were examined, and growth of FCoV, demonstrable by PCR, was obtained in most cases, some of which were FCoV antibody-negative. The conclusion reached was again: most healthy cats living in catteries with a past history of FIP are persistently infected with FcoVs [6]. The important finding of this biologically meaningful analysis was that the isolated viruses were of the 'non-cultivable' subtype I.

From the FCoV carrier state to FIP

What leads from infection to disease, from the chronic FCoV carrier state to FIP? This question will be asked by anybody who hears the classical anamnesis of a kitten obtained from a bona-fide breeder, kept in isolation from other cats that succumbed to FIP some weeks after the purchase.

The key pathogenic event in FIP is the infection of monocytes and macrophages. We formerly thought that avirulent FCoV strains remain confined to the digestive tract and would not spread beyond the intestinal epithelium and regional lymph nodes while virulent strains would disseminate to other organs via blood-borne monocytes. This idea can no longer be sustained, in view of the PCR results in healthy cats quoted above - the difference must rather be a quantitative one. In vitro, the virulence of FCoV strains was indeed correlated with their ability to infect cultured peritoneal macrophages. When strains were compared, however, the avirulent ones infected fewer macrophages and produced lower virus titres than virulent strains. Moreover, the avirulent strains were less able to sustain viral replication and to spread to other macrophages. This is no black-and-white phenomenon, rather a gradual transition, as the course of FIP is not uniform.

There is ample evidence for an involvement of the immune system in the pathogenesis of FIP. Humoral immunity is obviously not protective. FCoV-seropositive cats that are experimentally infected with FIPV often develop an accelerated, fulminating course of the disease, leading to the 'early death' phenomenon mentioned above. Clinical signs and lesions develop earlier, and the mean survival time is dramatically reduced as compared to seronegative cats. Direct evidence for the involvement of antibodies was obtained by transfusion of purified IgG from cat FCoV-antisera into cats, which indeed developed accelerated FIP upon experimental challenge. We also know, which antibodies are the killers: when vaccinia virus recombinants expressing single gene products were used to immunize cats, 'early death' occurred only in the group that had seen the spike (S) protein before.

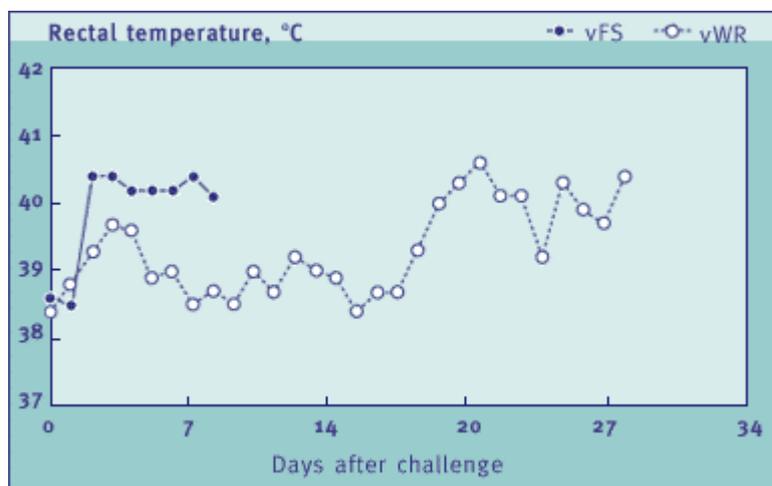


Fig. 8 The 'early death' phenomenon seen in cats that had been immunized with the S protein of feline coronavirus expressed by a vaccinia virus recombinant; the control animal had been vaccinated with the 'empty' vaccinia virus vector. Upon challenge with an FIP-producing coronavirus, these cats show the typical biphasic temperature rise and fatal course; the disease took more than two weeks, whereas the animals with antibodies to S succumbed within a week.

Most authors consider the vascular and perivascular lesions in FIP to be immune-mediated, but there is uncertainty about the actual pathogenetic mechanism. At least some vascular injury may be attributed to immune-mediated lysis of infected cells: FIPV-infected white blood cells were detected in the lumen, intima and wall of veins and in perivascular locations. Furthermore, inflammatory mediators such as cytokines,

leukotrienes and prostaglandins that are released by infected macrophages could play a role in the development of the perivascular pyogranulomata. These products could induce vascular permeability changes and provide additional chemotactic stimuli for neutrophils and monocytes. In response to the inflammation, the attracted cells may release additional mediators and cytotoxic substances; the monocytes would also serve as new targets for FIPV. The end result would be enhanced local virus production and increased tissue damage.

Other observations point towards an immune complex (ICX) pathogenesis. Deposition of ICX and subsequent complement activation is thought to cause an intense inflammatory response that may extend across blood vessel walls. The resulting vascular damage would permit leakage of fluid into the intercellular space and eventually lead to the accumulation of thoracic and abdominal exudate. The morphologic features of the vascular lesions (necrosis, polymorphonuclear cell infiltration associated with small veins and venules) strongly indicate an Arthus type reaction. The lesions contain focal deposits of virus, IgG and C3. Moreover, complement depletion and circulating ICX were demonstrated in cats with terminal FIP. In a horizontal study of experimentally infected cats, first clinical signs were accompanied by increased C3 concentrations in the plasma; subsequently antibody titres and circulating ICX increased with a concomitant decrease of complement concentrations. At the time of death, maximum ICX and minimum C3 concentrations were measured.

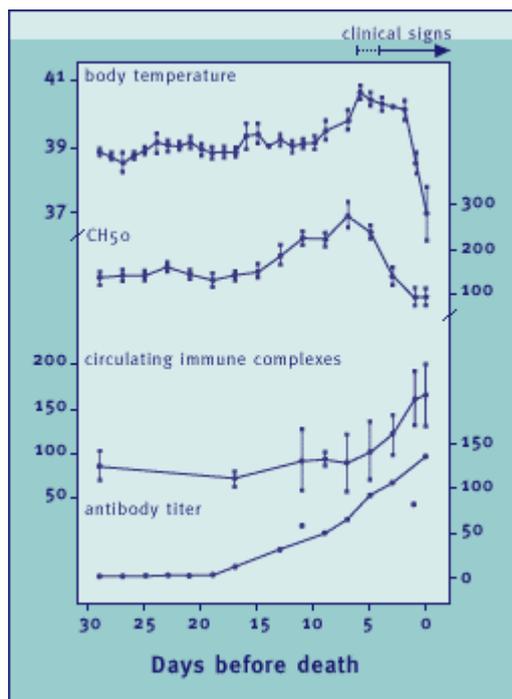


Fig. 9 Relationship between the clinical course of FIP, as represented by body temperature (upper curve), complement levels (middle), and concentrations of antibodies and immune complexes (lower curves) in the plasma. While the latter two parameters rise steadily, C3 levels are plummeting before death, indicating complement activation.

Although FIP viruses do not infect T-cells, depletion and programmed cell death (apoptosis) was observed in lymphoid organs of infected cats. Apoptosis was mediated by the ICX present in the serum and ascitic fluid of diseased cats and affected only activated T-cells, including lymph node cells, but not unstimulated T-cells. This hitherto unrecognised mechanism of T-cell suppression may operate not only in FIPV infection but also in other ICX diseases [5].

The fatal scenario thus may be as follows: a kitten is born, suckled by its seropositive queen and protected by colostral antibody from infection during the first few weeks. As

the maternal antibodies wane, mucosal protection ebbs away and during an episode of maternal FCoV shedding the kitten is infected. A bout of diarrhoea and an occasional sneezing may be the only signs this has happened. It now develops an active immunity, but not a sterilizing one in most cases: virus and antibodies continue to co-exist in the kitten's organism, and an efficient cell-mediated immunity keeps infected macrophages and monocytes in check. In a small, socially stable cat community this animal can live happily ever after.

Problems emerge when our kitten is experiencing any situation of stress, which we want to equate with immune suppression. Infection with the feline leukaemia or immunodeficiency viruses would be the most unmistakable immunosuppressive event, but density (numbers of cats per surface unit), geographic change (displacement into a new environment) and other territorial factors (e.g. change in group hierarchy, dominance) are becoming more and more important - in view of the declining prevalence of retrovirus

infections. The failing immune surveillance allows the coronaviral quasispecies cloud of mutants to expand, and more macrophage-tropic mutants emerge in this stochastic process. Amongst them are some that reach high titres and outcrowd the moderate ones. This is the point when immune pathogenesis starts.

Laboratory tests - are they useful?

The diagnosis of FIP has been covered in many textbooks and articles; in our view, the algorithm developed in the Zurich laboratory is still the best clinical guideline [10]. Although it includes titres in the decision tree, these constitute only a minor factor. A negative serologic result would not invalidate the diagnosis based on clinical and blood chemistry data.

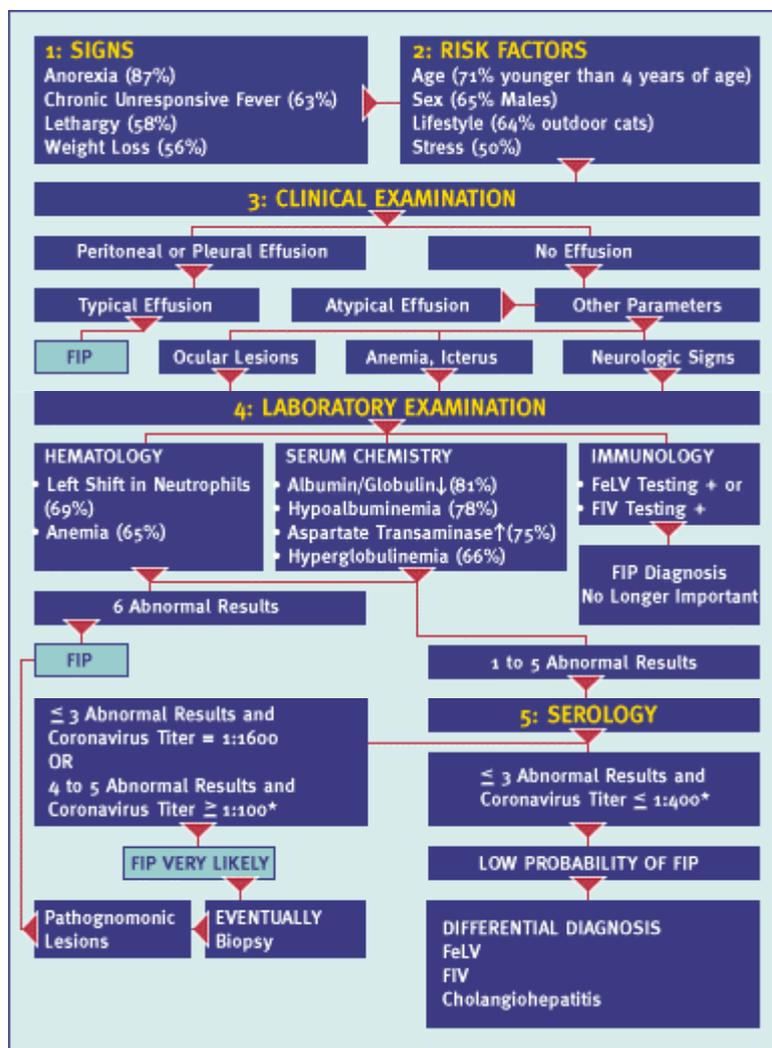


Fig. 10 Algorithm used to facilitate the diagnosis of FIP

In the absence of clinical signs, serology is of no use for the prognosis in individual cats. A statistical correlation indeed exists between antibody titres and post-mortem confirmation of FIP three months after testing. However, about 40% of the animals with titres of

How does the other face of that coin look? About 12% of the cats with titres < 100 still developed FIP in the observation period. Based on these data, one in eight owners would

have been sent home with the erroneous information that nothing will happen to his cat. Serology does not distinguish between harmless and FIP-inducing FCoV mutants, it only shows past - and in many cases still ongoing - infection. Any seropositive cat may succumb to FIP, irrespective of the titre. We now can explain this statistical correlation between high titers and poor prognosis: expansion of the coronavirus quasispecies cloud obviously not only provides much genomic material with the increased probability for FIP-inducing mutants to occur, it also provides the large antigenic mass to induce high levels of antibody. However, FIP-inducing mutants can always occur, also at low replication levels (with low antibody production), though with a lesser likelihood.

On the other hand: an uninfected cat - which is not synonymous with a seronegative cat - will not develop into a case of FIP. This may sound as a truism, nevertheless some consideration is justified. In the Hanover cattery study, a few seronegative kittens shed FCoV in both faeces and plasma, some in faeces only and others in plasma only. In concreto: 86% positive animals were detected using PCR, while serology showed only 71% positives. However, not a single seropositive animal tested PCR-negative [10]. The question must be asked whether there is a place for diagnostic and prognostic laboratory testing for FIP at all. There are presently no diagnostic assays available - neither in-practice tests nor assays performed in the research laboratory - that would distinguish between virulent (FIPV) and avirulent FCoV variants. Also the 'novel' PCR formats touted by some firms do not keep this promise, irrespective of the claims. We have reasons to believe that discriminatory assays based on the molecular properties of the variants will not be feasible, perhaps not even possible. However, there is a future for tests based on the evidence of immunological changes in an animal developing FIP.

Both serology and PCR are able to detect infected cats, with different sensitivity, and are invaluable for the management of catteries. They can be used for monitoring the success of the quarantine and 'early weaning' programmes, for controlling the specified pathogen-free and the coronavirus-free status of catteries. Especially PCR could be useful for monitoring individual animals to be introduced into FCoV-free catteries.

A promising approach to controlling FIP - based on isolation of litters after early weaning - has been developed by the Glasgow group [1] - but it is laborious, requires the dedicated cooperation of cat owners and has no veterinary appeal. Incidentally, other studies performed under similar conditions showed only marginal effects [7]. Another possibility is the removal of strong shedders from a multi-cat society. These can now be recognized by using the TaqMan technique: for a reliable characterization of the shedding pattern it is sufficient to test four faeces samples taken at weekly intervals. Strong shedders can be identified under field conditions and separated from the group, thereby decreasing infection pressure for the remaining cats. It remains to be shown whether this approach will work. However, common sense suggests that in conjunction with other measures (keeping cats in small groups, without contact between groups, frequent cleaning of litter boxes, introduction of new cats only after quarantine and PCR testing etc.) the elimination of strong shedders might be useful.

The seronegative catteries established through any control programme must of course be protected against re-introduction, and the live temperature-sensitive vaccine could prove useful for this purpose - if it indeed did not induce antibodies, thereby compromising a serology-based test-and-isolation programme. Also, persistence and recrudescence of the vaccine virus might then be studied. Still much must be learned about this most enigmatic infectious condition in veterinary medicine, feline infectious peritonitis.

References

1. Addie, D. D. and Jarrett, J. O. (1992). A study of naturally occurring feline coronavirus infections in kittens. *Vet. Rec.* 130, 133-137.
2. Egberink, H. F., Herrewegh, A. P. M., Schuurman, N. M. P., van der Linde-Sipman, J. S., Horzinek, M. C., and de Groot, R. J. (1995). FIP, easy to diagnose? *Vet. Quart.* 17, 24-25.
3. Eigen, M. (1993) Viral quasispecies. *Scientific American*, July ;269(1):42-49
4. Fehr D., Holznagel E., Bolla S., Hauser B., Herrewegh A.A., Horzinek M.C., Lutz H. (1997) Placebo-controlled evaluation of a modified live virus vaccine against feline infectious peritonitis: safety and efficacy under field conditions. *Vaccine.* 15: 1101-1109.
5. Groot, R.J. de and Horzinek, M.C. (1995) Feline infectious peritonitis. In: *The Coronaviridae* (Siddell, S.G. ed.) pp. 293-309. Plenum Press, New York.
6. Gunn-Moore D.A., Gruffydd-Jones T.J., Harbour D.A. (1998) Detection of feline coronaviruses by culture and reverse transcriptase-polymerase chain reaction of blood samples from healthy cats and cats with clinical feline infectious peritonitis. *Vet. Microbiol.* 62: 193-205.
7. Gut M., Leutenegger C, Huder J., Pedersen N., Lutz H. (1999) One-tube fluorogenic reverse transcription-polymerase chain reaction for the quantitation of feline coronavirus. *J. Virol. Methods* 77, 37-46
8. Haagmans, B.L., Egberink, H., Schijns V.E.C.J. and Horzinek, M.C. (1996) Apoptosis and T-cell depletion during feline infectious peritonitis. *J.Virol.* 70, 789-793.
9. Herrewegh, A.A.P.M., de Groot, R.J., Cepica, A., Egberink, H.F., Horzinek, M.C., and Rottier, P.J.M. (1995) Detection of feline coronavirus RNA in feces, tissue, and body fluids of naturally infected cats by reverse transcriptase PCR. *J.Clin.Microbiol.* 33, 684-689.
10. Herrewegh, A.A.P.M., Mähler M., Hedrich, H.J., Haagmans, B.L. Egberink, H., Horzinek, M.C., Rottier, P.J.M. and de Groot, R.J. (1997). Persistence and evolution of feline coronavirus in a closed cat breeding colony. *Virology* 234, 349-363
11. Pedersen, N. C. (1987). Virologic and immunologic aspects of feline infectious peritonitis virus infection. *Adv. Exp. Med. Biol.* 218, 529-550.
12. Poland, A.M., Vennema, H., Foley, J.E. and Pedersen, N.C. (1996) Two related strains of feline peritonitis virus (FIPV) isolated from immunocompromised cats infected with a feline enteric coronavirus (FECV). *J.Clin.Microbiol.* 35, 258-262.
13. Rohner. M. (1999) Bestimmung der Ausscheidungskinetik von feline Coronaviren unter Feldbedingungen. Inaugural-Dissertation Vet.Med., Zürich (1999).
14. Rohrer, C., Suter, P.F. and Lutz, H. (1993) Die Diagnostik der feline infektiösen Peritonitis (FIP): retrospektive und prospektive Untersuchungen. *Kleintierpraxis* 6, 379-381.
15. Vennema, H., Poland, A., Floyd Hawkins, K., and Pedersen, N. C. (1995) A comparison of the genomes of FECVs and FIPVs and what they tell us about the relationships between feline coronaviruses and their evolution. *Feline Pract.* 23, 40-44.