

## Unravelling the Secrets of Feline Infectious Peritonitis—SOTAL

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### *State of the Art Lecture*

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Dr.Horzinek has published over 250 articles in scientific journals and over 30 books, monographs, handbook and CD-ROM articles. He has been editor or member of the editorial boards of over 10 scientific journals in the Netherlands, Belgium, Great Britain, Germany, Austria, France, and Italy. Presently he is virology Editor-in-Chief of *Veterinary Microbiology*, an Elsevier journal.

He is founding member of the European Society of Feline Medicine (UK), a scientific society, and of the Gesellschaft für Kynologische Forschung (Germany), a fund-raising initiative for veterinary research. In May 2000, he started *Veterinary Sciences Tomorrow*, an electronic current awareness journal.

Feline coronaviral polyserositis, as it should be properly termed, is the fatal "tip of the iceberg" of a common infection of cats with a group of ubiquitous viruses. Coronaviruses are common pathogens found in mammals and birds, notorious for containing the largest of all RNA genomes. One out of 10,000 nucleotides is changed in any round of RNA genome replication; 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.

**Although generally associated with acute, self-limiting enteric and respiratory infections, coronaviruses can persist in infected animals. We have shown this to be true for feline coronavirus (FCoVs), which normally cause only mild enteric infections, and which occur in almost any cattery in Western Europe and America. The low-virulence "enteric" FCoV and the disease-causing FIPVs are closely related genetically; we think that the latter are virulent variants of the former, which arise in individual FCoV-infected hosts. This means that no two cases of FIP are caused by the same virus, and that horizontal transmission, i.e., cat-to-cat transfer is rather the exception than the rule.** On the basis of *in vitro* neutralization tests FCoVs can be allocated to serotypes: type I is prevalent in Europe and found in most fatal cases of FIP, type II may be more common in other parts of the world (e.g., Japan). The latter viruses are a showcase of viral evolution—they arise de novo through RNA recombination, during which genetic information from the canine coronavirus is incorporated into FCoV type I genomes.

To study viral evolution during chronic infection, the FCoVs sampled from individual cats were characterized. Phylogenetic comparisons indicated that they form clades (closely related clusters) and are likely to have originated from single founder infections. Each cat harbours a distinct FCoV quasispecies

with immune selection (antigenic drift) occurring during chronic infection. Blood samples from healthy cats of different breeds from different catteries—some of which were FCoV antibody-negative—were shown to contain FCoV, as demonstrated by PCR. The important finding of this biologically meaningful analysis was that the isolated viruses were of the “non-cultivable” subtype I.

All available data support a model in which chronic carriers maintain endemic infections in cat societies. Virtually every kitten born in a breeding facility becomes infected, probably from its queen, 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.

The key pathogenic event in FIP pathogenesis is the infection of monocytes and macrophages. The difference between avirulent and virulent FCoVs is probably 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.**

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, cytokines, leukotrienes and prostaglandins could play a role in the development of the perivascular pyogranulomas. These products could induce vascular permeability and provide 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.

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.

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 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 the kitten is under stress, to be equated with immune suppression.** Infection with the feline leukaemia or immunodeficiency viruses would be the most unmistakable immunosuppressive event, but population 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 would 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.

In the absence of clinical signs, serology is of no use for the prognosis in individual cats. **A statistical correlation exists between antibody titres and post-mortem confirmation of FIP three months after testing. However, about 40% of the animals with titres of < 300 do develop FIP, and of those with titres exceeding 1000 only about one half succumb; in other words, about half of the tested animals that remained healthy showed the same high titer values as the cats at risk. Tossing a coin would have given a similar result.** On the other hand, 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/her cat. **Serology simply cannot 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 titer.** 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, but in one cattery study, 86% of infected animals were detected using PCR, while serology gave only 71% positive results. However, not a single seropositive animal tested PCR-negative.

**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 tests in use nor assays performed in the research laboratory—that would distinguish between virulent and avirulent FCoV variants. In addition, the “novel” PCR formats touted by some firms do not keep this promise, irrespective of the claims.** There are 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 evidencing immunologic 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” programs and the coronavirus-free status of catteries. Especially, PCR could be useful for monitoring individual animals to be introduced into FCoV-free catteries.

A control approach based on isolation of litters after early weaning has been studied, but its effects could not be verified by other workers. Another possibility is the **removal of strong shedders from a multi-cat society. These can now be recognized by using a real-time PCR (the TaqMan technique); for a reliable characterization of the shedding pattern, it is sufficient to test four feces samples taken at weekly intervals (Hans Lutz, Zürich, personal communication). 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.

For References see an extensive review at

[http://www.vetscite.org/cgi-bin/pw.exe/vst/reviews/index\\_1\\_0800.htm](http://www.vetscite.org/cgi-bin/pw.exe/vst/reviews/index_1_0800.htm)