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Recommendations from workshops of the second international feline coronavirus/feline infectious peritonitis symposium

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Summary In August 2002, scientists and veterinarians from all over the world met in Scotland to discuss feline coronavirus (FCoV) and feline infectious peritonitis (FIP). The conference ended with delegates dividing into three workshops to draw up recommendations for FCoV control, diagnosis and treatment and future research. The workshops were chaired by the three authors and the recommendations are presented in this paper.

Recommendations for control of FCoV in catteries (Pedersen)

Due to time limits, the working group decided to concentrate on breeding and rescue catteries rather than veterinary practices, shows or boarding catteries as recommendations for the former would apply to the latter. We are using the generic term 'feline coronavirus' for a common RNA-containing virus in accordance with the guidelines set out in the Fifth International Symposium on Coronaviruses (Laude, 1994). Feline coronavirus (FCoV) is comprised of two closely related biotypes: (1) a ubiquitous form present in virtually all large multi-cat environments which leads to seroconversion and causes very little disease, known as feline enteric coronavirus (FECV) and (2) a much less common mutant form of FECV that has gained the ability to replicate in macrophages, and causes feline infectious peritonitis known as FIP virus (FIPV). Both biotypes exist in at least two strains (types I and II) with large numbers of genetic variants.

FIP is the major consequence of feline coronavirus infection, and because FIPV occurs as a mutant of the common FCoV, control of FIP must be directed first at control of its parent virus, and should that fail, at the FIPV itself.

Early weaning and isolation

Isolation of queens 2–3 weeks prior to parturition, strict quarantine of queen and kittens, and early weaning at 4–6 weeks of age is one means to prevent FCoV infection. This procedure is based on the findings that some queens do not shed the virus, some queens will stop shedding after several weeks if not re-exposed, and that even if they do shed, very young kittens have maternal resistance to the virus (Addie and Jarrett, 1992). Therefore, if you can prevent outside infection, you should be able to remove the kittens from the queen before they can be infected and then continue to raise them clear of the infection. Early weaning and isolation is not

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just a good idea for the control of FCoV, but also for the control of feline calicivirus, feline herpesvirus, *Bordetella bronchiseptica*, *Microsporum* spp. and the many enteric infections to which kittens are susceptible.

Although straightforward in concept, isolation of queens and early weaning is not as simple as it may seem. The procedure requires quarantine rooms and procedures that absolutely ensure that new virus does not enter. It also works best when the isolated queens are not shedding FCoV, when they are shedding low levels, or when they can clear the infection early after being isolated. The single factor that most assures these conditions is the number of animals. The success of early weaning and isolation in FCoV control depends on effective quarantine and low numbers of cats in the household, preferably under 5 or 6. If there are less than 5 or 6 cats, the chances of there being high or persistent shedders is low. Also, human abodes do not easily allow for adequate quarantine space for large numbers of queens and kittens and the time and money required to maintain quarantine goes up in proportion to the number of queens and litters under quarantine. As examples of environment and cat numbers, all kittens under 8 weeks old submitted to a USA shelter were FCoV negative (Pedersen et al., 2003). These were kittens that largely came from one-queen homes. In contrast, a Swiss study in large catteries demonstrated viral infection of kittens as young as 2 weeks old (Lutz et al., 2002). It is clear that low FCoV exposure will delay infection, while high exposure can overcome maternally derived immunity at an early age.

There are two essential downsides of isolation and early weaning. The first is that it is not easy to do and will fail if conditions are not proper. Second, some breeders believe that early weaning exacts a social price on the kittens. In recognition of both concerns, the group recommended that early weaning not be undertaken without careful consideration. FCoV-free households would not be required to undertake routine isolation and early weaning. Where kittens are isolated with their queen, extra care must be taken during the 2–7 weeks-of-age period to socialise the kittens. The success of early weaning should also be measured, and not continued in situations where it is not working. Kittens that have been successfully reared free of the FCoV should be antibody negative at 12 weeks of age. If they are antibody positive, it means that they have been infected with the virus.

Even if kittens can be raised free of FCoV, it is clear that they may become infected sooner or later. The virus is very widespread, even among outdoor cats, and it is easily carried on clothes, hair, hands, shoes, etc. (Pedersen et al., 1981). Therefore, the objective of isolation and early weaning should not be to prevent infection forever, but to delay it. It is known that immunity to FIPV does not develop until around 16 weeks of age (based on experience with Primucell® vaccine (Pfizer) (reviewed by Pedersen et al., 1995)). We also have anecdotal evidence that FIPV infection in shelter and cattery kittens occurs in the first 2 months or so of life, even though the actual disease may not appear outwardly for many weeks, months, and sometimes years.

Measurement of antibody titres and viral load

FCoV serology (also known as FIP serology) can be of some value, but only if it is performed accurately and expressed as an endpoint titre (Lutz et al., 2002), For instance cats with very low titres (1:25 or below) are often shedding no or low levels of virus (Addie and Jarrett, 2001), and will frequently stop shedding when isolated. Cats with high titres (1:400) are almost always shedding high levels of virus. Some of these cats will stop shedding upon isolation, and this will be demonstrated by a decrease in their titre to low or negligible levels. If a cat is persistently shedding virus, the titre will always remain high. If laboratories cannot offer accurate antibody testing, than the alternative is for commercial laboratories to make available quantitative RT–PCR to measure viral load. PCR based tests would be a direct measure of virus shedding. Veterinarians would be supplied with a faecal swab in a tube and a bar code. This would allow for accurate submission of samples.

Genetic markers in the cat

We know that three groups of FCoV shedders exist: (1) those that shed high levels of virus all of the time (about 10–15%), (2) those that seem to be immune to the virus and never shed (less than 5%), and (3) those that continuously lose and re-acquire the infection (about 70–80%) (Addie and Jarrett, 2001; Foley et al., 1997). Do high shedders or resistant cats have genetic markers for either state? Susceptibility to FCoV infection is likely to be different to susceptibility to the mutant FIPV. We know that the heritability of FIP is about 50%; susceptible cats being approximately twice as likely to develop FIP as other cats (Foley and Pedersen, 1996). That is why we do not recommend breeding

cats that have thrown kittens that later developed FIP. This would be especially true of toms, which can sire so many more kittens and therefore have a greater genetic influence on the bloodline.

Genetic markers of the virus

Are some FCoV strains more likely to mutate and thus cause FIP? We see some households without any cat deaths, despite endemic FCoV, while other households suffer many cases of FIP.

Vaccination

A FCoV vaccine may well be different from an FIPV vaccine, just as immunity to the two biotypes of the virus may differ. However, doubt was expressed about the possibility of ever developing a successful vaccine to the non-mutated form of FCoV (or FECV), because no vaccine can work better than natural infection. Most infected cats develop immunity, but the immunity disappears when the virus is controlled and the cats are then reinfected. Most cats are repeatedly infected with the same strain of FCoV, as well as by different strains (Addie et al., 2003). Panleucopenia vaccines work well because most cats in nature recover from the infection. Where hosts do not have good immunity, we often do not have good vaccines, e.g. feline calicivirus.

Shelters

Forty percent of young cats in the USA are now coming from shelters. Previously people acquired kittens from newspaper advertisements and word of mouth. Pedersen et al. (2004) found that admission into a rescue cattery resulted in high levels of shedding of feline calicivirus, herpesvirus and coronavirus. All of these viruses can cause long term consequences in an infected cat. Shelters need to optimise facilities and husbandry so they can be cleaned easily and minimise virus spread. It is essential to decrease viral load and stress levels in shelters.

Recommendations for diagnosing FIP (especially with regard to RT–PCR tests) and treatment (Paltrinieri)

Serology and RT–PCR

At the present time, FIP cannot be diagnosed solely by serology or on a positive RT–PCR test. In particular, no specific data regarding the pathogenic role of some mutated genes or proteins, detectable by RT–PCR or serology, have been published in independent peer reviewed scientific journals. The diagnosis of FIP is based on the history of the animal, the history of the disease signs, on gross clinical abnormalities, and a number of suggestive (but not specific) abnormal laboratory findings. Immunohistochemistry to identify viral proteins in macrophages within lesions can be used on tissues taken at biopsy or necropsy. Positive immunohistochemical staining of macrophages within lesions is considered the most definitive test for FIP. However, the possibility of detecting replicating FCoVs within circulating monocytes by RT–PCR was presented at this meeting and looks promising (Simons et al., 2002). Based on the assumption that only mutated FCoV can replicate within monocytes, this test or other future tests based on biologic behaviour of mutated FCoVs, might have a high diagnostic significance. More detailed descriptions of diagnostic tests are given below.

Recommended tests for diagnosing FIP

The most important tests for FIP are not laboratory, but rather historical. Most cats with FIP are from 6 months to 3 years of age, come from shelters or catteries, and show signs of cyclical antibiotic resistant fevers and specific physical manifestations depending on the form of the disease and location of lesions. A second tier of test findings include characteristic analysis of peritoneal or pleural effusions, elevated white blood cell counts with neutrophilia and lymphopenia, elevated globulin levels, and non-regenerative anaemia of chronic disease and hypoalbuminemia, and elevated fibrinogen. Laboratory tests, such as the serology and RT–PCR should comprise a third tier of diagnostics. Because a wide range of tests is quite expensive, it is prudent to start with basic tests first and add additional procedures only if preliminary testing justifies them. For these reasons we recommend starting with a laboratory approach only when the clinical signs are strongly suggestive of FIP and keeping in mind a list of possible differential diagnoses. This might help to choose the best panel of tests to apply to your case.

Analysis of the effusion

In the case of suspect effusive FIP, the analysis of the effusions remain the best diagnostic method, although it can be supported by other clinicopathological changes. In particular, protein and globulin determination, cytology and bacterial cultures should be performed. These tests might strongly support the diagnosis of FIP, when high

proteins and/or globulin concentrations are found in a sterile effusion with cytologic signs of a non-specific inflammatory process. In any case, they will rule out septic effusions and neoplasia (mainly lymphomas), but might not be enough to differentiate FIP from, for example, cholangiohepatitis. The detection of FCoVs in the effusion is the only conclusive test in these cases. To do this, immunocytologic techniques (immunofluorescence, immunohistochemistry) are preferable to the detection of FCoV genome by RT–PCR: although RT–PCR might easily detect FCoV in the effusions (Kita et al., 2002), as previously stated, it is a very sensitive technique and can detect any small amount of virus that might extravasate from blood to the effusion during every inflammatory process in cats with circulating FCoVs. In contrast, immunocytology detects only large amounts of virus and, moreover, allows identification of macrophages as the cells carrying the FCoVs. A positive result using these techniques can thus confirm the diagnosis of FIP, while an eventual negative result does not exclude the disease (Hartmann et al., 2002). In these cases, as in dry forms, the detection of other clinico-pathological changes is needed to support the clinical diagnosis of FIP.

Non-effusive FIP

In dry forms, a list of possible differential diagnoses must also be considered to suggest the best diagnostic approach. It is not possible in this report, to list all possible differential diagnoses, due to the extreme variability in clinical signs detectable in dry forms. This list, however, should include any possible cause of fever of unknown origin (FUO), uveitis, neurological alterations, hepatic or renal failure. The panel of tests to be used should be decided based on these symptoms and should always include a complete CBC (non-regenerative anaemia, neutrophilic leukocytosis and in particular, lymphopenia might have a high diagnostic value for FIP), the determination of the albumin/globulin ratio, eventually followed by a serum protein electrophoresis in the case of high globulins (α_2 and γ-globulins are expected to be elevated during FIP), and the measurement of α_1-acid glycoprotein levels (high concentrations of this protein, although not specific, might be strongly suggestive of FIP; Duthie et al., 1997). Although none of the above mentioned changes is per se suggestive of FIP, the presence of multiple alterations in cats with symptoms suggestive of FIP might highly increase the probability of correctly diagnosing the disease. Other tests might also be considered: in pure neurologic forms, for example, diagnostic imaging can exclude the presence of intracranial tumours, and antibody titre in CSF can be evaluated and compared to those in blood; a high CSF/blood ratio might be detected during FIP, based on the assumption that antibodies are produced within the CNS but the results of this test must be carefully considered, since alterations of the blood–brain barrier (BBB) are often present during FIP. The presence of a BBB damage can be excluded by measuring the serum:CSF ratio of antibodies against other infectious agents (e.g. herpesviruses). The cost/benefit ratio of such a complicated panel of tests, however, strongly reduces its practical use. The detection of histologic lesions consistent with FIP has been considered the only conclusive test for FIP for a long time (Barlough and Stoddart, 1998) and the finding of viral antigen in the lesions using immunofluorescence or immunohistochemistry (again, RT–PCR, is too sensitive) allows further confirmation of the diagnosis. Unfortunately, surgical biopsies cannot be taken frequently during FIP, due to the poor general conditions of the affected cats. The probability of detecting histologic lesions or positive macrophages in ultrasound-guided tru-cut biopsies (TCB) or in fine needle aspiration biopsies (FNA) is very low and negatively correlated with the extension of the pyogranulomatous foci (Paltrinieri, manuscript in preparation). Based on the general health status, the clinician should then decide among the following three diagnostic approaches: expose the cat to the risk of anaesthesia and laparoscopy/laparotomy to obtain surgical biopsies and gather a conclusive diagnosis of FIP; perform a non-invasive bioptic technique (TCB, FNA) with the possibility of a false negative result; obtain only a presumptive diagnosis based on clinico-pathological changes. A presumptive diagnosis, however, would be not enough to subject the cat to any treatment.

In conclusion, the only conclusive diagnosis of FIP must be obtained by the detection of FCoVs within macrophages in the effusions or within the lesions detected in surgical biopsies. If such an approach cannot be followed, the presence of multiple clinico-pathological changes might support the clinical diagnosis of FIP in both wet and dry forms. Serology and RT–PCR are much more useful in the cattery management than in the diagnosis of the disease.

Recommendations on treatment of FIP

No therapies have been proved to be effective for FIP, and the use of alternative treatments and so-called immunosuppressive or immunomodulating drugs should be suspect. Although encouraging

results obtained using feline recombinant feline interferon have been presented at this meeting (Ishida et al., 2004) further data are needed before recommendation of extensive use of this treatment. The best treatment at the present time is to stage the disease and treat symptomatically. As long as the cat is eating, feeling relatively well, and not losing weight, affected animals should be fed a high quality diet and kept as stress free as possible. In contrast, if the cat is losing condition, is suffering from specific debilitating signs of the disease, and has a poor quality of life, treatment should be counselled against. Severely affected animals should then be euthanased, due to short survival expectation. Even in cats with mild initial disease signs, the ultimate mortality is over 95%. However, miracle cures do happen from time to time, and miracles cannot happen unless they are allowed time to happen.

Recommendations for priority areas of future FCoV research (Addie)

1. In the absence of an effective vaccine, it was considered a priority to prevent cats becoming infected with FCoV at all. It was considered important to look at ways of minimising virus dose. Existing cat litters need to be checked for their ability to limit FCoV transmission by biocidal action and/or good clumping. The effect of flushing litter trays on FCoV spread needs to be investigated.
2. The ideal vaccine should protect against FIP, give good mucosal immunity to prevent infection and reduce virus shedding. Development of a therapeutic vaccine should also be considered, both to treat cats with FIP and to attempt to stop carrier cats from shedding. For the latter, it is essential to establish where the virus is in carrier cats (the ileum and colon are the most likely areas) so that immune clearance of virus from this area is taken into consideration in vaccine development.
3. The group was concerned about antibody dependent enhancement (ADE) being a laboratory artefact (Addie et al., 1995) and that experimental vaccines which might have worked perfectly well in the field had been rejected because in experimental infections they caused ADE. A reasonable challenge virus needs to be defined. The 79-1146 strain is probably not a good choice, since it is extremely virulent and also is a type II FCoV. The working group called for standardisation of vaccine challenge protocols worldwide, using a constant virus dose, strains more representative of natural infection (including types I and II) and natural exposure challenge (i.e. challenge not given parenterally). The virus dose threshold over which FIP develops would need to be established. A challenge virus stock should be made and stored in two or three locations worldwide and supplied from there to those working on novel vaccines.
4. The current belief is that cats with FIPV do not transmit the mutant virus to other cats (Vennema et al., 1998). Studies have shown that 40% or more of cats with FIP shed FCoV from their gut, but that the virus is of the intestinal type and will not cause FIP when given to susceptible kittens (Foley, J.E., and Pedersen, N.C., UC Davis, unpublished information). In contrast, virus taken from internal lesions readily induces FIP. The FIP causing virus is only present within macrophages in internal lesions, where it has no access to the outside. However, some investigators have seen 'outbreaks' of FIP, which can best be explained by an FIPV carrier. Although there are alternative explanations for such mini-epidemics, it is theoretically possible for cats with lesions in their kidneys or intestinal wall to shed FIPV in urine or feces.
5. More molecular work needs to be undertaken on the exact mutations that cause FCoVs to become FIPVs and how these mutations change the behavior of the virus:

   - to establish whether all FIPVs have 3c deletions
   - to define the functions of non-structural proteins 3a, b, c and 7a and b
   - to do a worldwide phylogenetic study so that future vaccines will cover as many natural FCoV strains as possible.

6. More work needs to be undertaken to grow the type I FCoV in cell culture. Different cell lines should be tried, and if that fails, the type I receptor needs to be found and cloned into a cell line.
7. More work is required to understand exactly what FIPV does in the infected macrophage. In addition, the cytokine profiles of naturally infected cats needs to be determined.
8. The phenomenon of resistant cats requires further investigation. Might it be possible to breed cats resistant to FCoV infection? Could resistant cats simply have been exposed very early in life (e.g. in the first week) and therefore have become immune tolerant?
9. Since the research community in FCoV is small, it is important that we exchange ideas more

often and work together. An email newslist will be established and the www.felinecoronavirus.com website will continue as a place where researchers can list available reagents.

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