Short Communications

Feline coronavirus in the intestinal contents of cats with feline infectious peritonitis

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THE question of whether or not cats with clinical feline infectious peritonitis (FIP) excrete the causative agent, feline coronavirus (FCoV), is important to the practising veterinary surgeon for two reasons. First, they have to know whether a cat with FIP in their surgery poses a hazard to other cats. Secondly, they may have to advise owners of a cat with FIP whether it is safe to obtain another cat while the affected cat is still alive (bearing in mind that cats with dry or non-effusive FIP can live for many weeks or months). In experimental infections, cats with FIP have been shown not to shed FCoV by the time they are clinically ill (Stoddart and others 1988). However, in this communication, the authors show that FCoV can be detected in the faeces and intestinal contents of cats which are naturally infected and suffering from FIP.

The most intractable obstacle to the complete understanding of the epidemiology of FIP has been the near impossibility of detecting FCoV in infected cats. Most attempts to isolate the virus in cell culture from cases of FIP, or from cats suspected of carrying the virus, have failed and only about 20 isolates have been made worldwide since the virus was identified over 30 years ago. The recent development of the reverse transcriptase-polymerase chain reaction (RT-PCR) to detect FCoV RNA, presumably representing whole virus, in faeces and body fluids of cats (Herrewegh and others 1995) may circumvent some of these problems and provide an opportunity to answer some of the outstanding questions about the transmission of FCoV. In this communication the results of a study to determine whether cats with naturally occurring FIP excrete FCoV, by examining their gut contents for the presence of FCoV RNA by RT-PCR, are presented.

Histopathological samples, blood, ascitic fluid and faeces or intestinal contents from cats suspected of having FIP were obtained on the day of death. FIP was confirmed by histopathology as previously described (Addie and others 1995). Samples of the contents of stomach, small or large intestine, or faeces were available from 23 cats with effusive FIP and five cats with non-effusive FIP. The samples were stored at –80°C until tested. FCoV was detected by RT-PCR (Herrewegh and others 1995). Faeces from a specific pathogen free (SPF) cat served as a negative, FCoV-uninfected control. In addition, material from three seronegative cats with lymphosarcoma, intestinal adenocarcinoma and feline parvovirus infection and a seropositive cat (anti-FCoV titre of 320) which died of cholangitis and had no histopathological evidence of FIP was tested. Anti-FCoV antibodies in plasma or ascitic fluid were measured by immunofluorescence (Addie and Jarrett 1992).

TABLE 1: Results of RT-PCR testing for FCoV in the gut contents of cats with histopathologically confirmed FIP

Cat	Sample	IFA titre	Type of FIP	PCR
1	intestinal contents*	20	effusive	–
2	stomach contents	40	effusive	+
3	SI contents	80	effusive	+
4	SI contents	160	effusive	+
	LI contents			+
5	LI contents	160	effusive	+
6	SI contents	160	effusive	–
7	faeces	320	non-effusive	+
8	SI contents	320	effusive	+
9	intestinal contents*	320	non-effusive	+
10	faeces	640	non-effusive	+
11	rectal swab	640	effusive	+
12	faeces	640	effusive	+
13	SI contents	1280	effusive	–
14	faeces	1280	effusive	+
15	LI contents	1280	effusive	–
16	faeces	1280	non-effusive	+
17	SI contents	1280	effusive	–
18	SI contents	1280	non-effusive	+
19	faeces	1280	effusive	+
20	faeces	>1280		+
21	faeces	>1280	effusive	+
22	SI contents	>1280	effusive	–
	1 month before death			–
	8 months before death			+
23	SI contents	>1280	effusive	+
24	stomach contents	>1280	effusive	–
	duodenal contents			–
	jejunal contents			–
	ileal contents			–
	caecal contents			+
	LI contents			–
25	LI contents	>1280	effusive	+
26	intestinal contents*	>1280	effusive	+
27	SI contents	>1280	effusive	+
28	stomach contents	>1280	effusive	+
	duodenal contents			+
	ileal contents			+
	jejunal contents			+
	caecal contents			+
	colon contents			+
	rectal contents			+
		Non-FIPs		
29	intestinal contents*		parvovirus	–
30	SI contents	0	lymphosarcoma	–
31	faeces	320	lymphocytic cholangitis	+
32	faeces	0	SPF	–
33	faeces	0	adenocarcinoma	–

SI Small intestine, LI Large intestine, *Exact area of collection unknown

As shown in Table 1, 22 of the 28 cats with naturally occurring FIP were positive for FCoV RNA by RT-PCR. Of the six cats which were negative, four of the samples tested were of small intestinal contents collected at post mortem examination, so that the question arose of whether FCoV was either not present in small intestinal samples or present but in too small quantities to detect (and possibly being concentrated further down in the bowel). Samples from all parts of the gut were available for only two cats, cats 24 and 28, and virus was detected only in the caecum of cat 24 and in

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all areas of the gastrointestinal tract of cat 28. Overall, at death, five of 12 small intestinal contents were negative, compared with only two of 15 large intestinal contents, faecal samples or rectal swabs. These proportions are not significantly different (P=0·18, Fisher's exact test).

All of the samples from cats with non-effusive FIP were positive. Control faeces from an SPF cat and faeces or intestinal contents from the three cats with lymphosarcoma, intestinal adenocarcinoma and parvovirus infection were negative.

It is clear from observations in the field, based on serology, that FCoV is transmitted from healthy carriers (Pedersen and others 1981, Addie and Jarrett 1992). The results of experimental infections with laboratory isolates of virus suggest that cats may excrete FCoV in the faeces for a limited period of time. Thus, cats experimentally infected with feline enteric coronavirus (FECV) strain 79-1683, shed virus for a period of only 14 to 17 days (Pedersen and others 1984). Furthermore, cats infected with an FIP-producing strain of FCoV shed virus in the faeces for an average of nine days but by the time they showed clinical signs, had stopped shedding (Stoddart and others 1988). However, experimental infections may not reflect the situation following natural infection, since recent results indicate that FCoV excretion from healthy cats can last for seven months or more (A. A. P. M. Herrewegh, M. Maehler, H. F. Egberink, H. Hedrich, P. J. M. Rottier, M. C. Horzinek, R. J. de Groot, unpublished observations).

The present results differ from those of experimental infections in which virus was not found by the time the cats died of FIP. There are several possible explanations for this difference. First, experimental infections may be different from natural infections in terms of virus dose and the immune response to infection which may influence both the pathogenesis of FIP and the shedding of FCoV. Secondly, it may be that by the time a cat has developed clinical FIP only very small amounts of virus are being shed which may be detectable by amplification of nucleic acid but not by less sensitive techniques of virus isolation or electron microscopy. Indeed, virus detected in the stomach or small intestinal contents of some of the present cats might never have reached the large intestine and been excreted. Thirdly, the RT-PCR can only detect viral RNA which may not necessarily be contained in infectious virus particles, although this is considered to be very unlikely because of the lability of naked RNA. However, if the virus was lacking its spikes it would be uninfectious.

Several of the present findings are of significance to practising veterinary surgeons. First, the detection of FCoV RNA in the seropositive cat with cholangitis, but not FIP, shows that cats with conditions other than FIP may excrete virus, as may healthy seropositive cats (Herrewegh and others 1995). Therefore, positive detection of FCoV in faeces by RT-PCR does not indicate that a cat is suffering from FIP. Secondly, the finding of virus in the faeces of cats with FIP suggests that these cats may be contagious (although it has not been demonstrated that transmissible virus was detected in this study). Therefore, should owners of cats with clinical FIP be advised to isolate the sick cat from other cats in the same household? FCoV is very contagious and it is likely that, by the time cats present with FIP, all of the cats in the household will be infected, so that isolating the sick cat might be pointless. However, it would be wise to isolate cats with clinical FIP from cats with no known previous exposure to FCoV and not to introduce new cats into the household until after the cat with FIP has died.

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Stillbirth/perinatal weak calf syndrome: serological examination for evidence of *Neospora caninum* infection

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A STILLBIRTH/perinatal weak calf (SB/PWC) syndrome, characterised by the birth of calves which are either stillborn, born alive but do not breathe, or do not breathe for more than 10 minutes, has been reported in Northern Ireland (Smyth and others 1992). A recently reported bovine mortality survey found that 5·4 per cent of deaths in calves under one month old which were attended by a veterinary surgeon were due to this syndrome (Menzies and others 1996). In a survey of stillborn and perinatal weak calves, Smyth and others (1992) attributed some deaths to infectious, metabolic or traumatic causes. However, the cause of death remained undiagnosed in a substantial number of cases.

The apicomplexan parasite *Neospora caninum* was first recognised as a cause of bovine abortion in California (Anderson and others 1991, Barr and others 1991) and has subsequently been shown to have a worldwide distribution (Thornton and others 1991, Ogino and others 1992, Wouda and others 1992, Jardine and Last 1993). In order to examine the involvement of *N caninum* in the SB/PWC syndrome, sera from 73 such calves were examined for antibodies to *N caninum*. This short communication reports the results of this survey and discusses their significance.

The serum samples were collected from calves submitted to Veterinary Sciences Division for post mortem examination during 1993 and 1994. They were tested according to the manufacturer's protocol (VMRD, Washington), using an indirect fluorescent antibody test (IFAT) on Teflon-masked multiwell slides coated with *N caninum*-infected Vero cells. Bound antibody was detected using FITC-conjugated polyclonal rabbit anti-bovine immunoglobulin G (Nordic Immunology, Tilburg) at a dilution of 1/90. All slides were read using a x 40 oil immersion lens and incident ultraviolet illumination.

Samples were initially screened at a dilution of 1/320, as recommended by Paré and others (1995). Those showing specific apical fluorescence were considered positive and titrated in doubling dilutions until an end point was reached.

Of the 73 samples examined, four (5·5 per cent) had titres ≥1/320 to *N caninum*. Details of their individual end point titres are given in Table 1. All of the calves were full term.

Histopathological examination was performed on the liver and thyroid gland of all four *Neospora* antibody-positive calves, on the heart and lung of three of the four calves and on the brain of one calf (calf 4). The last had mild perivascular cuffing in the hindbrain and mild lymphocytic infiltration of the meninges.

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Notes