Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats

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The pattern of shedding of feline coronavirus (FCoV) was established in 155 naturally infected pet cats from 29 households over periods of up to five years. Viral RNA was detected in faeces by reverse-transcriptase PCR (RT-PCR), and plasma antiviral antibodies by immunofluorescence. The cats rarely shed FCoV in their saliva. Three patterns of FCoV shedding were observed. Eighteen of the cats shed virus continuously, so were persistent, and possibly lifelong, carriers; none of them developed feline infectious peritonitis. Fifty-six cats ceased shedding virus, although they were susceptible to reinfection, and 44 shed intermittently or were being continuously reinfected. Four of the cats were resistant to infection. Seventy-three per cent of the virus shedding episodes lasted up to three months and 95 per cent up to nine months. There was a correlation between shedding and antibody titre but the cats could remain seropositive for some time after they had ceased shedding virus. One-off testing for FCoV by RT-PCR is inappropriate. Identification of long-term carriers requires that a positive result be obtained by RT-PCR on faecal samples for at least eight consecutive months. A cat should be shown to be negative over five months, or to have become seronegative, to ensure that it has ceased shedding virus.

FELINE coronavirus (FCoV) is a common infection of domestic cats. Virus transmitted in the faeces of carrier cats is believed to be responsible for maintaining the infection in cat populations (Foley and others 1997, Herrewegh and others 1997). A small proportion of infected cats develops feline infectious peritonitis (FIP), a fatal condition produced by an inappropriate immune response to the virus. The diagnosis of both FIP and FCoV infection has proved to be difficult, partly because the type of FCoV that is common in cats cannot be detected by isolation in cell culture. The diagnosis of FIP has been improved by the adoption of various algorithms (Lutz and others 1995, Duthie and others 1997) although a definitive diagnosis still relies on the histology of lesions. In the diagnosis of FCoV infection, the detection of antibodies has proved to be useful for preventing the transmission of the virus and for eliminating the infection from households of cats (Gonon and others 1995).

The introduction of a reverse-transcriptase PCR (RT-PCR) to detect the viral genome (Herrewegh and others 1995) has raised expectations that it could be used to improve diagnosis by positively identifying carriers of the virus and cats that are free of the infection. However, sequential tests on individual FCoV-infected cats showed that towards the end of the infection they excreted the virus in their faeces intermittently (Herrewegh and others 1997). A single positive or negative result might therefore be misleading. This paper describes a study designed to establish criteria by which RT-PCR could be used to detect carrier cats and cats which were free of infection.

MATERIALS AND METHODS

One hundred and fifty-five cats from 20 multicat and nine single-cat households in which FCoV was naturally endemic were followed for up to five years. Blood samples were taken into heparin at intervals of three to 12 months and the plasma was tested for anti-FCoV antibodies by indirect immunofluorescence (IF) (Addie and Jarrett 1992). The cat owners or their veterinary surgeons took samples of faeces or rectal swabs and saliva swabs from the cats at intervals of not less than a month. The samples of faeces were tested by a RT-PCR targeted to the highly conserved 3′ non-translated region of the viral genome (Herrewegh and others 1995), and FIP was diagnosed by its clinical signs and characteristic histopathology (Addie and others 1995).

Stringent precautions were taken to prevent the contamination of samples with FCoV. The amplification achieved with RT-PCR is so great that contamination with even a few molecules of viral RNA can be detected. In one household, the results from the faeces of seven seronegative animals were positive on 12 occasions, suggesting that the samples had been contaminated at source. As a result, rectal swabs were adopted as the sample of choice. In the laboratory, standard measures were taken to avoid contamination. Even so, there was always the possibility that contamination had occurred, and the inclusion of a test to detect antibodies was a useful check for this possibility.

RESULTS

The type of sample that was most appropriate for the detection of viral RNA was investigated. The two forms of faeces that were available were those which were shed into litter trays, and rectal swabs. Rectal swabs had the advantage of being less likely to be contaminated with faeces from other cats, but faecal samples had the advantage that they could be stored for future investigations. The choice of which sample to obtain from each household depended on the likelihood of cross-contamination, and whether or not the cat was free ranging, making faecal samples inaccessible.

In experimental FCoV infections, the virus was found to be shed in saliva (Stoddart and others 1988). Therefore, at the beginning of the study, saliva and faeces samples were obtained and subjected to RT-PCR. The results of 144 comparisons of saliva and faeces are shown in Table 1. Of 60 cats with positive faecal samples, 54 gave salivary swabs which were negative for FCoV RNA, whereas of 84 cats with negative faecal samples, only four gave positive saliva samples. It was concluded that saliva was not a useful sample with which to determine the shedding status of a cat, and the monitoring of saliva was discontinued. None of the cats shed FCoV in the saliva on more than one occasion. Of the 10 positive saliva results, five were recorded in the first sample taken from the cat as it entered the study, suggesting that salivary excretion might occur early in infection. In two cats, a positive saliva result preceded faecal shedding. In another cat, the positive

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TABLE 1: Comparison of sample of saliva and faeces for the detection of shedding of feline coronavirus

Number of faeces samples	Number of saliva samples Positive	Negative
Positive	6	54
Negative	4	80

saliva result was recorded 28 months after it joined the survey and appeared to coincide with its reinfection, because the cat's antibody titre increased and it resumed shedding FCoV in its faeces.

Patterns of FCoV shedding in faeces

The study observed the same outcomes of natural FCoV infection that have been described by Foley and others (1997) and Herrewegh and others (1997) (Table 2). Eighteen became chronic carrier cats and shed virus continuously; four cats were resistant, never shed virus and possibly did not seroconvert; 56 became transiently infected, shed virus for a time, seroconverted, ceased shedding virus and eventually became seronegative; in 44 cats in multicat households it was not possible to differentiate re-infection from intermittent shedding. In 19 cases it was uncertain whether the cats had not been exposed or were resistant, and in 14 cases too few samples were obtained to determine their category.

Carrier cats Carrier cats were defined as those that shed FCoV at every test and remain infected for life. To define a carrier cat, it must be determined for how long a cat can shed FCoV and then subsequently eliminate it. A carrier cat would then be one that shed virus continually for longer than that period. The shedding periods of the cats which eliminated FCoV were therefore examined. Fifty-six cats eliminated FCoV infection, but 12 of them later became re-infected, some of them more than once. Seventeen of the cats were never found to shed virus and their infection was only apparent because they were seropositive and subsequently became seronegative, and two were tested by RT-PCR on only two occasions. Seventy-eight virus-shedding episodes from the remaining 37 cats are recorded in Table 3; 73 per cent of them lasted less than four months, 87 per cent for less than six months and 95 per cent for less than nine months. Therefore, for a cat to be suspected of being a lifelong carrier, it should be shown to shed virus continually for at least nine months.

In the group which did not eliminate the virus but did not shed it continually, one cat shed the virus continually for 26 months and then showed a negative RT-PCR test. This result suggests that to establish that a cat was definitely a lifelong FCoV carrier, it would have to shed the virus for longer than

TABLE 3: Periods of shedding of feline coronavirus (FCoV) by 37 cats that eliminated the FCoV infection

Longest period of shedding (months)	Number of virus-shedding episodes	Percentage of total
1	45	57·7
2	4	5·1
3	8	10·3
4	2	2·6
5	9	11·5
6	2	2·6
7	2	2·6
8	2	2·6
9	0	0
10	3	3·8
11	1	1·3
Total	78	

TABLE 2: Patterns of shedding of feline coronavirus (FCoV) in the faeces of 155 cats

Category	Number of cats
Carrier cats	18
Cats which eliminated FCoV	56
Resistant cats	4
Possibly unexposed, possibly resistant	19
Intermittent shedders or reinfected cats	44
Too few samples to determine	14
Total	155

26 months. However, this cat may have been being re-infected by other cats in the same household. Therefore, in this survey, a carrier cat was defined as a seropositive cat that shed FCoV for at least nine months.

Nineteen of the cats did not shed FCoV and remained seronegative. Since their owners segregated them from virus-shedding cats, for example, cat 13 and cat 14 in the household shown in Table 4 were kept in a different house from known FCoV shedders, it was impossible to know whether they had been exposed. There remained 136 cats that were definitely exposed to FCoV and 18 (13 per cent) of them were definite carriers. Shedding of virus was detected in these cats at each sampling for periods of 10, 13, 13, 13, 18, 18, 21, 29, 31, 33, 35, 35, 39, 40, 43, 44, 46 or 48 months, respectively, and was continuing at the end of the survey, or when the cat died. In a previous study, before RT-PCR was available (Addie and others 1995), one cat was believed to be a carrier and this suspicion was confirmed in the present survey. It was therefore likely that it had been a carrier of FCoV for 12 years. A further three cats were suspected of being carriers, but in these cases virus shedding was not monitored for long enough or sufficiently frequently to be certain: they were positive for at least four, seven and seven months, respectively.

TABLE 4: Antibody titres to feline coronavirus (FCoV) and reverse-transcriptase PCR (RT-PCR) results in a household that successfully eliminated FCoV infection by segregating the positive and negative cats

Cat	Mar 96	May 96	July 96	Oct 96	Aug 97	Jan 98	Apr 98	Jun 98	Aug 98	Jan 99	Nov 99	Feb 2000	Aug 2000
1	1280	1280	320	640	20	10							
	–	+	+	–	–	–							
2	1280	>1280	640	>1280	640	160		320					
	–	+	+	+	–	–		ND					
3	640	640	640	640	160	20		40					
	+	+	+	+	–	–		ND					
4	640	320	160	80	ND	0							
	+	+	+	–		–							
5	80	80	40	20	20	0		10					
	+	–	–	+	–	ND		ND					
6	160	40	20	40	20	0		10					
	–	–	+	–	–	–							
7	40	20	10	0	10	10		10					
	+	–	–	–	–	–							
8	>1280	320	160	160	80	80		40		40			
	–	–	–	–	–	–							
9	1280	1280	>1280	>1280	1280	160		80					
	+	+	+	+	–	–							
10	640	640	640	1280	>1280	>1280	ND		>1280	>1280	ND	ND	
	+	+	+	–	+	+	+				+	+	
11	10	20	0	20									0
	–	–	–	–									ND
12	0	0	0	0									0
	–	–	–	–									ND
13				0									
				–									
14				0									
				–									

Figures represent antibody titre. Shading indicates when a cat was moved to another household
– Negative RT-PCR result from rectal swabs, + Positive RT-PCR result, ND Not done

TABLE 5: Correlation between the shedding of feline coronavirus determined by reverse-transcriptase-PCR (RT-PCR) and antibody titre determined by indirect immunofluorescence

Antibody titre	RT-PCR results Positive	Total	Percentage positive
<10	10	81	12
10	3	29	10
20	9	42	21
40	6	23	26
80	13	33	39
160	23	47	49
320	60	85	70
640	76	98	77
1280	51	65	78
>1280	27	36	75

Fate of FCoV carrier cats One carrier cat died under anaesthetic and another from a cerebellar haemorrhage. A possible carrier was diagnosed clinically as having FIP, but the diagnosis was not confirmed by histopathology. The remaining cats are still alive and well at the time of writing, apart from a tendency to chronic diarrhoea in some of the cats. Although the number of cats is small, it is clear that being a carrier does not predispose a cat to the development of FIP. The kitten of one carrier cat died of FIP.

Transient FCoV shedding Most of the cats were transient shedders; they ceased shedding virus in their faeces and their anti-FCoV antibody titres declined to less than 10 (Table 4). However, 24 cats that eliminated the virus had an intermittent pattern of virus shedding. This pattern could have been due to true intermittent virus excretion (only three of the cats were kept in isolation), or could have been due to reinfection, because 21 of the cats could have been exposed to virus from in-contact cats. In addition, 34 cats that had not yet eliminated virus at the time of writing had an intermittent pattern of virus shedding. To identify the cats that had eliminated the infection, it was therefore necessary to define a minimum period of non-shedding after which it was very unlikely that a cat would shed the virus again. In defining this period, one problem was that cats that appeared to have eliminated the virus could become reinfected, probably owing to a decline in immunity, as described below.

Intermittent shedders were difficult to distinguish from cats which became re-infected and they could be identified only if they were kept indoors in isolation from cats which were potential virus shedders. Seven cats were in this situation and in these cats, 10 periods of intermittent shedding were recorded. There were three periods during which no virus was detected for up to one month, three periods of up to two months, one period each of up to three, four and five months, and one period of between four and seven months.

Seven cats (including two of the seven above) appeared to be in the process of eliminating the virus, in that they gave negative results on RT-PCR, had declining levels of antibodies, and gave a single positive result before they finally ceased shedding and became seronegative. An example is cat 5 (Table 4). The intervals between the penultimate and final positive RT-PCR result in the seven cats were three to four months in two cats, three to seven months, seven to 10 months, nine to 11 months, 10 to 11 months and 10 to 17 months. (These cats were not all tested monthly, which meant that it was impossible to be precise about the exact interval until their final positive result. The first figure is the interval between first and last negative tests, in cat 5 this is three months. The second figure is the interval from the last known positive test to the subsequent positive test, in cat 5 this is seven months: March to October.) These cats had antibody titres of 0, 20, 20, 20, 20, 80 and 640 at the time of their last positive test. Although in most cases five consecutive negative monthly faecal tests would indicate that a cat had cleared FCoV infection, these terminal bursts of virus shedding could occur.

Many more negative periods without apparent shedding occurred in the cats that lived in groups and did not eliminate FCoV infection. When a cat remained seropositive throughout, it was not always possible to determine whether it was intermittently shedding virus, or being re-infected. Cats tested negative by RT-PCR for one to two months on 22 occasions (27 per cent), three to seven months on 36 occasions (44 per cent), and for over seven months on 24 occasions (29 per cent). In seven cases, a return to shedding was almost certainly due to re-infection.

Eleven of the 29 households completely eliminated FCoV infection from their cats; seven of them were single-cat households. An example of a multicat household in which virus shedding ceased as a result of intervention is given in Table 4. Seventeen months into the survey only one cat, cat 10, still shed FCoV. It was confined to a single room and kept in strict isolation with barrier nursing precautions. After five months of isolation, it was evident that it was a carrier and it was rehomed to a single-cat household for its own welfare.

In two households FCoV was almost eliminated, with all the cats except a single carrier ceasing to shed virus. In one household of seven cats, the cats appeared to be re-infected by the carrier cat. In the second household of 10 cats, the carrier cat died before the the others were reinfected, and the source of the reinfection therefore remained unknown. The cats often became reinfected after their antibody titre waned, suggesting that humoral immunity might play a role in their resistance to infection.

Two cats that eliminated FCoV died during the survey; one was euthanased for an unknown reason, and the other had an alimentary lymphosarcoma.

Resistant cats Four of the cats (2·9 per cent) that were exposed to the virus showed no signs of being infected. (Percentages are based on 136 known FCoV exposed cats, since 19 survey cats were isolated from FCoV shedders within the same homes and may never have been exposed.) They differed from the uninfected cats in that they were known to be mixing in the household freely with infected cats, but were resistant to, rather than immune from, FCoV. They never shed the virus and either remained seronegative, or had a very low antibody titre.

Correlation of FCoV shedding and antibody titre

The RT-PCR results obtained in the same or subsequent month as an antibody measurement are shown in Table 5. In general, the higher the antibody titre, the greater was the risk that a cat would be shedding FCoV. The percentage of cats shedding virus increased steadily up to an antibody titre of 640, at which it levelled off at around 75 per cent. Several seronegative animals tested positive for virus in their faeces, but in one household in which 12 positive results were obtained from the faeces of five seronegative cats, the results were considered to be due to contamination of the samples; these results are not included in Table 4. There remained 10 samples from seronegative cats that were shedding virus. One cat gave three positive RT-PCR results when its antibody titres were 20, 10 and 0. No other cat with an antibody titre of 10 or less when the antibody titre was declining was positive by RT-PCR.

The longest period for which a cat remained seropositive but did not shed virus was 25 months, it had an anti-FCoV antibody titre of 1280 which had decreased to 80, 25 months later. A second cat had an antibody titre of 1280, stopped shedding the virus five months later, and only became seronegative after a further 12 months. A third cat stopped shedding virus but has remained seropositive for 20 months.

Fate of the cats

Four adult cats and two kittens developed FIP. Two of the adults were from the same household and the two kittens from another. Since 136 adult cats became infected or were exposed to FCoV, this gives a mortality rate of 3 per cent to FIP. One cat became infected 18 months into the survey and died six months later. It came from a household where an attempt was being made to segregate the infected and uninfected cats, as in the household illustrated in Table 4. One cat shed virus continually for four months and the other three cats shed intermittently before they died. All four adult cats had antibody titres of 1280 or greater when they died. Seventeen cats died for reasons other than FIP.

Chronic diarrhoea in FCoV shedding cats

Four carrier cats, and three which were intermittent FCoV shedders, suffered from chronic diarrhoea. One cat was euthanased as a result of this condition. Another died under anaesthetic whilst being biopsied to establish a diagnosis. A histological examination provided no explanation for the chronic diarrhoea.

DISCUSSION

This study of virus shedding required appropriate samples from pet cats in order to detect FCoV RNA by RT-PCR. Initially saliva and faeces samples were compared. The virus was detected in only 10 of 144 (7 per cent) saliva swabs. The virus is relatively fragile, and it is possible that the samples were too degraded by the time they reached the laboratory to be truly representative. However, saliva from one cat known to be a carrier was examined within two hours and was also negative. The FCoV in faeces survived for at least 10 days in the mail in a condition to be detectable by RT-PCR. It was therefore concluded that monitoring the shedding of FCoV in saliva would not be a good indicator of the infectious status of a cat, and that samples of faeces or rectal swabs would be more useful. There was an indication that FCoV was shed in saliva early in an infection, in agreement with the results of experimental infections when FCoV was often shed in saliva before being detected in the faeces (Stoddart and others 1988).

The RT-PCR and antibody assays taken together gave a more reliable indication of the infectious status of a cat than either assay alone. Cats that ceased shedding virus, and showed a decline in antibody titre to 10 or less, could confidently be said to have eliminated the infection. As observed by Herrewegh and others (1997), some cats in isolation shed the virus intermittently; some cats which had been negative for up to seven months spontaneously began to shed the virus again. This finding makes it impossible to interpret a single virus shedding result. However, most cats stopped shedding virus after less than five months and for practical purposes a cat with five consecutive negative RT-PCR tests can be considered to have eliminated FCoV, whether or not they remain seropositive. This is an improvement on using IF antibody test results alone, because some cats can remain seropositive for over 25 months without shedding the virus.

Fifty-six of the cats (41·2 per cent) conclusively eliminated FCoV infection. Of these cats, 31 per cent were never found to shed FCoV, and were assumed to have been infected because they were seropositive. In 95 per cent of cases, FCoV was shed for less than nine months and it therefore seemed reasonable to suppose that most cats that shed FCoV for nine months or more were carrier cats. However, one cat shed FCoV continually for 26 months, ceased shedding for two months, and then started shedding the virus again. This result indicated that cats might stop shedding virus after longer periods. Other cats in the same household may have reinfected this cat. What is required is a definitive marker of carrier cats so that they can be kept apart from susceptible animals.

Eighteen (13 per cent) of the infected cats became healthy, persistent carriers of the virus. Some of them shed virus intermittently, or possibly became reinfected, before they became carrier cats. None of the 18 cats developed FIP over periods of 10 to 48 months. Four of the carrier cats suffered from chronic diarrhoea.

Four of the cats appeared to resist FCoV infection, despite living in households where the virus was endemic. One possible explanation for this resistance is that the virus may be unable to infect the cells of cats if they lack an appropriate receptor for FCoV. The cellular receptor for FCoV may be aminopeptidase N, a metalloprotease of the intestinal brush border (Tresnan and others 1996, Hegyi and Kolb 1998, Kolb and others 1998, Tresnan and Holmes 1998). A mutant form of the receptor might render the cells resistant, as has been described for human immunodeficiency virus (Michael and others 1998). Alternatively, the resistance might be due to a type of immune response that cannot be detected at present. These cats warrant further investigation because they may provide an insight into ways of protecting cats against FCoV infection and FIP.

The shedding of the virus correlated well with the cats' antibody titres, as measured by IF, although some seronegative animals were positive by RT-PCR. This result was unexpected because the kittens of seronegative queens did not become infected (Addie and Jarrett 1992), implying that seronegative cats are not infectious. It may be that because the cats were in households where FCoV was endemic, and virus-shedding cats were detected before they seroconverted, as described by Harpold and others (1999). This appeared to be the case in four cats which became seropositive on the next sample. After experimental oral infection with FCoV, antiviral antibody first appears in the serum after seven days, and the titres take over 18 days to peak (Stoddart and others 1988). A second possible explanation of the results is that contamination of the samples with virus led to false positive results in the RT-PCR. This might have occurred because virus from one sample of faeces contaminated another at the source, as was clearly the case in one household. Another site of contamination could be the laboratory. Here, despite each part of the RT-PCR procedure being conducted carefully in different rooms, and the inclusion of appropriate controls at each stage, contamination did occur very occasionally. It was clearly beneficial that the cats were monitored by both the antibody assay and RT-PCR, because the results of the former gave warning of potential problems in the latter. A third possible explanation for the disagreement between the results of the antibody assay and the RT-PCR is that the results of the antibody assay were incorrect. This explanation is considered to be the least likely because the IF test is very robust and the reproducibility of the results obtained in the test is well established.

In practical terms, this study established useful guidelines for the use of RT-PCR in detecting the shedding of FCoV by cats. Saliva was shown to be of little use as a sample because the virus was shed only transiently in the saliva of only a few of the infectious cats. The virus was detected much more reliably in faeces. To identify carriers of FCoV, continuous virus shedding should be demonstrated over a period of more than nine months. To show that a cat has stopped shedding FCoV and may be mixed safely with other cats, at least five consecutive monthly negative faecal tests should be obtained, or the cat should be shown to have become seronegative by IF. Because the RT-PCR is prone to both false positive and false negative results, it is important that it be used in conjunction with the IF antibody assays. Most important of all, serial tests should be applied because the interpretation of a single test is very uncertain.

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References

ADDIE, D. D. & JARRETT, O. (1992) A study of naturally occurring feline coronavirus infection in kittens. *Veterinary Record* **130,** 133-137

ADDIE, D. D., TOTH, S., MURRAY, G. D. & JARRETT, O. (1995) The risk of feline infectious peritonitis in cats naturally infected with feline coronavirus. *American Journal of Veterinary Research* **56,** 429-434

DUTHIE, S., ECKERSALL, P. D., ADDIE, D. D., LAWRENCE, C. E. & JARRETT, O. (1997) Value of α1-acid glycoprotein in the diagnosis of feline infectious peritonitis. *Veterinary Record* **141,** 299-303

FOLEY, J. E., POLAND, A., CARLSON, J. & PEDERSEN, N. C. (1997) Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments. *Journal of the American Veterinary Medical Association* **210,** 1307-1312

GONON, V., ELOIT, M. & MONTEIL, M. (1995) Evolution de la prevalence de l'infection a coronavirus felin dans deux effectifs adoptant des conduites d'élevage differentes. *Recueil de Medecine Veterinaire* **171,** 33-38

HARPOLD, L. M., LEGENDRE, A. M., KENNEDY, M. A., PLUMMER, P. J., MILLSAPS, K. & ROHRBACH, B. (1999) Fecal shedding of feline coronavirus in adult cats and kittens in an Abyssinian cattery. *Journal of the American Veterinary Medical Association* **215,** 948-951

HEGYI, A. & KOLB, A. F. (1998) Characterization of determinants involved in the feline infectious peritonitis virus receptor function of feline aminopeptidase N. *Journal of General Virology* **79,** 1387-1391

HERREWEGH, A. A. P. M., DE GROOT, R. J., CEPICA, A., EGBERINK, H. F., HORZINEK, M. C. & ROTTIER, P. J. M. (1995) Detection of feline coronavirus RNA in feces, tissue, and body fluids of naturally infected cats by reverse transcriptase PCR. *Journal of Clinical Microbiology* **33,** 684-689

HERREWEGH, A. A. P. M., MAHLER, M., HEDRICH, H. J., HAAGMANS, B. L., EGBERINK, H. F., HORZINEK, M. C., ROTTIER, P. J. M. & DE GROOT, R. J. (1997) Persistence and evolution of feline coronavirus in a closed cat-breeding colony. *Virology* **234,** 349-363

KOLB, A. F., HEGYI, A., MAILE, J., HEISTER, A., HAGEMANN, M & SIDDELL, S. G. (1998) Molecular analysis of the coronavirus-receptor function of aminopeptidase N. *Advances in Experimental Medicine and Biology* **440,** 61-67

LUTZ, H., FEHR, D., ROHRER, C. & SUTER, P. F. (1995) Current knowledge on FIP from a European perspective; current concepts of the disease, current and future research. Proceedings of the AAFP Symposium on Feline Infectious Diseases. Washington DC. pp 22-27

MICHAEL, N. L., NELSON, J. A, KEWALRAMANI, V. N, CHANG, G., O'BRIEN, S. J., MASCOLA, J. R., VOLSKY, B., LOUDER, M., WHITE, G. C. 2nd, LITTMAN, D. R., SWANSTROM, R. & O'BRIEN, T. R. (1998) Exclusive and persistent use of the entry coreceptor CXCR4 by human immunodeficiency virus type 1 from a subject homozygous for CCR5 delta32. *Journal of Virology* **72,** 6040-6047

STODDART, M. E., GASKELL, R. M., HARBOUR, D. A. & GASKELL, C. J. (1988) Virus shedding and immune responses in cats inoculated with cell culture-adapted feline infectious peritonitis virus. *Veterinary Microbiology* **16,** 145-158

TRESNAN, D. B. & HOLMES, K. V. (1998) Feline aminopeptidase N is a receptor for all group I coronaviruses. *Advances in Experimental Medicine and Biology* **440,** 69-75

TRESNAN, D. B., LEVIS, R. & HOLMES, K. V. (1996) Feline aminopeptidase N serves a receptor for feline, canine, porcine, and human coronaviruses in serogroup I. *Journal of Virology* **70,** 9669-9674

Aetiology of reduced milk ejection in cows after transport and the use of a long-acting analogue of oxytocin for prophylaxis

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Milk flow was recorded in 21 cows for three days after they were admitted to a large animal hospital. When the spontaneous flow of milk had stopped, a physiological dose (1 iu) of oxytocin was administered intravenously. Five of the cows were, in addition, treated with 0·35 mg of a long-acting analogue of oxytocin (carbetocin) one hour before the first milking after they were admitted. In the 16 cows not treated with carbetocin, only about 30 per cent of the total milk yield was released spontaneously on the first day, and the injection of 1 iu of oxytocin released approximately another 60 per cent of the total milk yield. On the second day, the proportion of the total milk yield released spontaneously increased and the fraction released after the injection of 1 iu oxytocin decreased. In contrast, the five cows treated with carbetocin released on average 94 per cent of the total milk yield spontaneously during the first milking.

TEAT stimulation is known to be important for the release of oxytocin and milk let-down before cows are machine milked (Mayer and others 1984, Gorewit and Gassman 1985, Merrill and others 1987, Pfeilsticker and others 1996). Oxytocin is released from the pituitary gland via a neuroendocrine reflex arc (Crowley and Armstrong 1992) and causes milk to be released from the alveoli, a prerequisite for the availability of milk for machine milking. Without increased oxytocin concentrations, only the milk which is stored within the teat and the gland cistern can be removed (Bruckmaier and Blum 1998).

However, despite normal teat stimulation, milk let-down may be inhibited, and in such cases, milk yields are markedly reduced despite well-filled udders and the absence of any udder or systemic diseases. The problem occurs mainly in primiparous cows but occasionally affects pluriparous or postpuerperal cows (Mielke and Brabant 1963, Schulz and Brabant 1970, Bruckmaier and others 1992, Schulz and Petzold 1998).

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Use of a reverse-transcriptase polymerase chain reaction for monitoring the shedding of feline coronavirus by healthy cats

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Notes