# PAPERS & ARTICLES

# Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus and feline coronavirus in stray cats sent to an RSPCA hospital

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A total of 517 stray cats at an RSPCA veterinary hospital were tested for feline leukaemia virus (FeLV), feline coronavirus (FCoV) and feline immunodeficiency virus (FIV). The prevalence of FeLV was 3-5 per cent in all the cats, 1-4 per cent in healthy cats and 6-9 per cent in sick cats. FeLV positivity was associated only with disease of non-traumatic origin. Antibodies to FCoV were present in 22-4 per cent of the cats, and their prevalence was significantly higher in cats over two years old and in feral/semiferal cats. The prevalence of antibodies to FIV was 10-4 per cent in all the cats, 4-9 per cent in healthy cats and 16-7 per cent in sick cats. The prevalence of antibodies was significantly higher in entire males and neutered males than in females, in cats over two years old compared with younger cats, and in cats suffering disease of non-traumatic origin rather than in healthy cats or cats suffering only from trauma. Sex, age and health status were each independently highly associated with FIV antibodies.

STRAY cats are often managed by rehoming, and it is therefore important to study the prevalence of disease among them and the occurrence of any high-risk groups. Many studies have examined the prevalence of feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus (FCoV) in selected groups of cats in the UK (Gruffydd-Jones and others 1988, Hosie and others 1989, Addie and Jarrett 1992b). Inevitably, such studies have tended to be biased towards privately owned cats which have been taken to a veterinary surgeon.

FeLV appears to be spread by 'friendly' cat contact, usually licking and grooming. It is found most commonly in younger colony cats. In the general UK population, its prevalence is considered to be around 18 per cent in sick cats and 5 per cent in healthy cats (Hosie and others 1989). Conversely, FIV infection appears to be spread by 'unfriendly' cat contact, usually from bites. It is thought to be most common among older, free-roaming, entire male cats. Hosie and others (1989) found that the prevalence in the UK was 19 per cent in sick cats and 6 per cent in healthy cats. Both diseases are therefore likely to be significant among stray cats. The prevalence of antibodies to FCoV in the UK is thought to be between 39 and 84 per cent in pedigree cats (Addie and Jarrett 1992b, Sparkes and others 1992) and between 14 and 19 per cent in domestic cats (Horzinek and Osterhaus 1979, Addie and Jarrett 1992b). High titres of FCoV are more often found in crowded groups of cats (Horzinek and Osterhaus 1979), the virus being transmitted faecally; FCoV seropositivity and hence feline infectious peritonitis may therefore be less common among stray cats.

This paper reports the results of testing for these viruses among 517 cats submitted to the RSPCA as strays from Birmingham and its suburbs, and also considers the occurrence of risk groups within this population and the association of their virus status with specific illnesses.

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## MATERIALS AND METHODS

### Cats

The cats tested were brought in to or picked up by the RSPCA collection service at Barnes Hill, Birmingham, as stray cats, that is cats with no carer or owner. A total of 517 cats were tested between August and December 1997, and they came only from Birmingham and its surrounding suburbs. To reduce sampling bias, every stray cat received by the RSPCA

hospital during this period was tested, whether it was healthy or ill, and every stray cat that could be assessed at the associated RSPCA rehoming cattery was also tested. Ninety-nine of the cats admitted during this period were not tested owing to their being acutely ill or for other reasons. The Birmingham RSPCA accepts all the stray cats received or collected by the RSPCA in the Birmingham area; the sample is therefore representative of stray cats without acute terminal disease, which are the cats which are most likely to be rehomed.

The age, sex, breed, tameness, health status, body condition, and illnesses suffered by each cat were recorded.

### **Data collection**

The details of each cat were recorded on a standard form and a blood sample was taken. Detailed records were kept by veterinarians on the cats' state of health when first examined and on any diseases they acquired while in the hospital or at the rehoming cattery. They were divided into four groups: cats that appeared healthy; cats that appeared healthy except for a recent traumatic illness, for example, road traffic accidents, acute fractures in healthy bone, lacerations, missing parts of or whole limbs or tails, witnessed accidents, collar injuries and ligament ruptures; cats suffering disease that was primarily non-traumatic, including gastrointestinal, haematological, urinary and respiratory disease, age-related disease, tumours, infection-based disease, central nervous system and ocular disease where no traumatic aetiology could be found - this group also included cats which might have suffered trauma but in which the primary problem was not traumatic; and cats in which the disease aetiology could not be differentiated between traumatic and non-traumatic.

The cats were classified as male entire, male neutered or female; the female cats were placed in one group because in most cases it could not be established whether they were neutered or entire. Age was recorded as kitten (under six months), six months to two years, two years to 10 years, or aged (over 10 years) as determined by an examination of the teeth. Breed was recorded as domestic (including domestic shorthairs and longhairs) or pedigree. The pedigree group included cats that may have been pedigree cross but showed strong pedigree characteristics. The cats were classified as tame, semiferal or feral, on the basis of consistent behavioural characteristics, but owing to the small numbers, the semiferal and feral cats were grouped together for statistical analysis. Body condition was recorded as average, underweight or overweight and was assessed by an examination of each cat's physical condition. The cats were also classified into four groups to describe whether they acquired new illnesses while in hospital: the cats that remained well or had an illness that was stable; cats that showed signs of a new illness not recorded at the initial examination; cats that had an illness that became worse; and cats that were unclassifiable because they remained in care for less than two days. All the cats were examined for identification and scanned for microchips.

### Sample collection and analysis

Blood samples were collected by jugular or cephalic venepuncture into heparinised tubes. They were tested for FeLV antigen and antibodies to FIV by the rapid immunomigration (RIM) assay test (Speedcat; Bioveto) according to the manufacturer's instructions. The assay detects FeLV antigen p27 and antibodies to FIV gp41. Most of the samples gave a clear positive or negative result, that is, a definite line was produced in the positive zone of the kit or it remained blank. However, a few samples gave a 'ghost' band which was considerably fainter than the control band; bands which were only slightly fainter than the control band were recorded as positive.

The remaining blood was posted without refrigeration to the Feline Virus Unit (FVU) diagnostic laboratory at the University of Glasgow where the samples were tested for FeLV by ELISA (Lutz and others 1983). All the positive samples and any in which the results of the RIM and ELISA differed were examined by virus isolation (Jarrett and others 1982). The samples were also tested for FIV by immunofluorescence as described by Pedersen and others (1987) and any result that did not agree with the RIM was retested by Western blot (Hosie and Jarrett 1990). Fourteen samples were not retested as there was not enough blood. The samples were tested for antibodies to FCoV by immunofluorescence as described by Addie and Jarrett (1992a). Any samples that showed non-specific immunofluorescence were retested by Western blot.

### **Statistical analysis**

Initially the results for each virus were cross-tabulated against the factors under consideration on a Microsoft Excel spreadsheet, and Fisher's exact probability test was used to screen these factors for further consideration. Univariate logistic regression was used to corroborate the cross-tabulated results and to provide an alternative interpretation of the results as an odds ratio (OR) of the effect of that factor.

Factors significant by the univariate analyses were included in multivariate analyses. To achieve a 'parsimonious' model a stepwise procedure was used to select the best subset of explanatory factors. In this way similar or similarly acting factors were excluded and only the most significant was retained. The multivariate logistic regression used a forward selection process. Only those factors whose Wald P values were less than 5 per cent were selected. This approach had the benefit of providing additional estimates of both P values and the 95 per cent confidence interval (CI) for the OR associated with each factor. Univariate and multivariate logistic regression was performed using SAS version 6.12 for Windows (SAS Institute). P values less than 0-05 were considered significant.

### RESULTS

### FeLV

Of the 517 samples tested at the FVU one gave an incomplete result and hence 516 samples were used for the analysis. Of these, 18 (3.5 per cent) were ELISA FeLV positive. For three samples, the results were discordant, with negative results by virus isolation and RIM but positive ELISA results; these results were classified as positive in the statistical analysis. A total of 515 FeLV results were complete by both the RIM and laboratory

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TABLE 1: Comparison of the results of the rapid immunomigration (RIM), ELISA and immunofluorescence (IF) tests for feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV)

	RIM I	ELISA positive	ELISA negative	9
Fell	v Positive	9	1*	
	Negative	5†	492	
(	Ghost (non-specific)	4*	4	
	RIM	IF positive	IF negative	IF non-specific
FIV	Positive	46	3	0
	Negative	3‡	455	15
(	Ghost (non-specific)	3	4∞	1*

\* ELISA and virus isolation (VI) results in agreement

<sup>†</sup> One sample retested VI positive, three retested VI negative

<sup>‡</sup> One sample retested Western blot (WB) positive

§ WB positive

<sup>∞</sup> Two samples retested WB negative

\* WB p24 antibodies

testing; eight of them were classified as RIM ghost results (Table 1).

The prevalence of FeLV was examined for each risk factor (Table 2). No correlation could be found between the cats' FeLV status and sex, age, breed, tameness, body condition or illness suffered while in hospital (Table 3). Non-traumatic illness was the only factor that showed a significant correlation with FeLV positivity (P=0.0025). Of the 18 cats that were FeLV positive, 13 had a non-traumatic illness, one was healthy and one had been in a road traffic accident. Of the three cats with discordant results, one was healthy, one had a non-traumatic illness and one had suffered a fractured jaw. OR analysis showed that the cats with non-traumatic illness had a six-fold higher risk of FeLV infection than healthy or traumatised cats, with a 95 per cent CI of 2 to 20.

### FIV

Of the 517 cats tested, all but two gave clear results on testing at the FVU. One of these gave a non-specific result by

TABLE 2: Prevalence of feline leukaemia virus (FeLV), and antibodies to feline immunodeficiency virus (FIV) and feline coronavirus (FCoV) among stray cats sent to an RSPCA hospital according to their sex, age, breed and other factors

Factor	Number of cats tested*	FeLV positive (%)	FIV positive (%)	FCoV positive (%)
Sex				
Female	236	2.5	3-4	20.7
Male entire	194	5.7	18-6	23.2
Male neutered 86		1.2	11.6	25.6
Age				
<6 months	95	2.1	4.2	11.5
6-24 months	133	3.0	3.8	18-8
24-120 months	218	5-0	14-2	27.5
>120 months	70	1.4	20-0	28-6
Breed				
Domestic	506	3.6	10.7	22.0
Pedigree	10	0	0	40-0
Tameness				
Tame	467	3.6	9-4	20-5
Feral/semiferal	49	2.0	20-4	40-8
Non-traumatic illness				
No <sup>†</sup>	285	1.4	4.9	22.4
Yes	204	6.9	16.7	22.1
Body condition				
Average	370	3.0	7-0	22-4
Overweight	19	5.3	21.0	0
Underweight 127		4.7	18·9	26-0
Subsequent illness <sup>‡</sup>				
New illness	47	4.3	14.9	25.5
None	331	3-0	8-1	20.2

\* Excluding one cat from FeLV analysis due to incomplete results

† Excluding 27 cats with illness of uncertain status

<sup>‡</sup> Excluding 138 cats of uncertain status

# TABLE 3: Univariate odds ratio with approximate 95 per cent confidence intervals (CI) and Wald P values, for levels of factors against the reference level of that factor for feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline coronavirus

Factor (reference level)	Level of factor	FeLV*	Ρ	FIV*	Ρ	FCoV*	Р
Sex (female)	Male entire	2.3 (0.9, 6.8)	0.11	6.5 (3.1, 15)	<0.0001	1.2 (0.7, 1.8)	0.53
	Male neutered	0.5 (0.02, 2.7)	0.46	3-8 (1-4, 10)	0-0071	1.3 (0.7, 2.3)	0.35
Age (<6 months)	6-24 months	1.5 (0.3, 11)	0.67	09 (02, 37)	0-88	1.8 (0.9, 4.0)	0.13
	24-120 months	2.5 (0.6, 16)	0.25	3.8 (1.5, 13)	0-014	2.9 (1.5, 6.2)	0.0024
	>120 months	0.7 (0.03, 7.2)	0-75	5.8 (2.0, 21)	0.0031	3.1 (1.4, 7.2)	0.0066
Tameness (tame)	Feral/semiferal	0.6 (0.1, 2.8)	0-57	2.5 (1.1, 5.1)	0.020	2.7 (1.4, 4.9)	0.0017
Non-traumatic illness (no)	Yes	5.7 (2.0, 20)	0-0025	2.9 (1.6, 5.3)	0.0003	10 (06, 15)	0.87
Body condition (average)	Over or underweight	1.6 (0.6, 4.3)	0.31	3.1 (1.8, 5.6)	0.0001	1.0 (0.6, 1.6)	0.95
Subsequent illness (no)	Yes	1.04 (0.92, 1.16)	0.50	1.07 (1.0, 1.14)	0.058	1.04 (0.99, 1.09)	0-14

\* OR and 95 per cent CI

immunofluorescence, a ghost result by RIM and had antibodies to FIV p24 by Western blot; it was a kitten and the result may have been due to maternal antibodies. Another sample was non-specific by immunofluorescence, negative by RIM and positive by Western blot. Both samples were included as positives in all the analyses. A total of 516 cats had complete RIM results for FIV; eight of them were ghost results (Table 1).

A total of 54 cats (10.4 per cent) had antibodies against FIV, and the prevalence of FIV was examined for each risk factor (Table 2). There was a significant correlation between FIV antibody-positive status and sex, age, health and body condition (Table 3). There was some correlation with tameness, but none with breed or subsequent illness, although this may have been affected by the small numbers in these groups. Female cats were significantly less likely to be FIV positive than male cats, especially entire males (P<0.0001). Entire males had a six-fold higher risk and neutered males a four-fold higher risk than females of being FIV positive. OR analysis showed that cats six to 24 months of age were as likely to have FIV antibodies as those under six months of age, but that cats aged two to 10 years were four times as likely to be FIV positive (P=0.014) and cats over 10 years old were six times as likely to be positive as cats less than six months of age (P=0.0031).

Cats with a non-traumatic illness were three times more likely to be FIV positive than healthy cats or cats suffering only trauma (P=0.0003). Cats over or under average body condition were also much more likely to be FIV positive; both overweight and underweight cats were three times as likely to be infected as cats in average body condition (P=0.0001). Feral and semiferal cats were at increased risk of FIV by a factor of 2.5 (P=0.02). All 10 pedigree-type cats were FIV negative, but the numbers were too small to draw any conclusions. There was no significant relationship between FIV infection and the development of illness while in RSPCA care (P=0.058) but this too may have been affected by the small numbers in this group.

Only two cats tested positive for both FIV and FeLV. A multivariate analysis was applied to determine whether the attributes that predicted the risk of FIV infection were independent of each other. Sex, age and non-traumatic illness were found

TABLE 4: Multivariate odds ratio with approximate 95 per cent confidence intervals and Wald P values for levels of factors against the reference level of that factor for feline immunodeficiency virus (FIV) and feline coronavirus (FCoV)

Factor (reference level)	Level of factor	FIV	Ρ	FCoV	Р
Sex (female)	Male entire	11 (4.7, 27)	<0.0001		
• • •	Male neutered	2.7 (1.0, 7.7)	0.047		
Age (<6 months)	6-24 months	1.0 (0.2, 4.0)	0.95	1.9 (0.9, 4.3)	0.10
<b>U</b> · · ·	24-120 months	3.6 (1.3, 13)	0.025	2.8 (1.4, 5.9)	0.0038
	>120 months	4.9 (1.3, 21)	0.022	3.2 (1.4, 7.4)	0.0057
Tameness (tame)	Feral/semiferal	2.5 (1.3, 4.7)	0.0037		
Non traumatic illness (no)	Yes	5 7 (2 0, 20)	0.0020		
Body condition (average)	Over or underweight	3·2 (1·5, 6·8)	0.0019		

to correlate independently with FIV infection (Table 4). After adjusting for the effects of age and non-traumatic illness, entire males had an 11 times higher risk of FIV infection than females, with a 95 per cent CI of 5 to 27 (P<0.0001).

#### FCoV

All 517 samples were tested for FCoV by immunofluorescence, and 116 (22.4 per cent) were seropositive. Non-specific fluorescence was found in six samples; of these, two were positive by Western blot and four were negative, and they were classified in the analysis accordingly. The prevalence of FCoV antibodies was examined for each risk factor (Table 2). Only age and tameness were associated with FCoV seropositivity, and all the other factors - FeLV or FIV infection, health, sex, breed, body condition, and subsequent disease - did not appear to be correlated with FCoV status (Table 3). Using OR analysis, cats six to 24 months of age were twice as likely as cats less than six months of age to have a positive titre to FCoV, although this difference was not statistically significant (P=0.13). Cats over two years of age were three times as likely as cats less than six months old to have a FCoV-positive titre (P=0.0024). Feral and semiferal cats were about three times as likely as tame cats to have a FCoV-positive titre (P=0.0017). By multivariate analysis, age and feral status were correlated independently with FCoV status (Table 4).

#### **Other data**

The clinical signs of the cats that were ill when first examined were recorded, and 101 different clinical signs or syndromes were observed. However, there were too few cats in each category for a useful statistical analysis. Nevertheless, there were some interesting relationships. Gingivitis was diagnosed in four cats, of which two were FeLV positive. Necrotising stomatitis with gingivitis was recorded in nine cats, of which four were FIV positive. Severe periodontitis affected five cats, of which four were FIV positive. Eight cats had tumours and two of them were FeLV positive. Old injuries, that is, previously healed major injuries, often fractures, were recorded in 10 cats, of which four were FIV positive and four were FCoV positive. Thirty-one cats had abscesses, of which 10 were FIV positive. Wounds affected by cutaneous myiasis were seen in five cats and, of these, three were FIV positive. Visible anaemia was recorded in 10 cats, three of which were FIV positive. Overt clinical signs of renal failure were present in 12 cats, four of which were FIV positive.

Thirty of the cats (5.8 per cent) were claimed by their owners. None of the cats had collar identification, and only five scanned positive for microchips.

# DISCUSSION

Most of the trends observed in this study of stray cats were similar to those found in previous studies of FIV and FeLV that concentrated on privately owned cats. The main difference was the apparently lower prevalence of FeLV; a positive FeLV result was recorded in 1.4 per cent of the healthy cats and 6.9 per cent of the sick cats, less than observed by Hosie and others (1989) and Shelton and others (1989) who found that 12 to 18 per cent of privately owned sick cats were FeLV positive. However, in Norway only 1.2 per cent of healthy owned cats and 2.2 per cent of sick cats were positive for FeLV (Ueland and Lutz 1992). This may be due to the more solitary lifestyle of stray cats, because FeLV is spread predominantly by close social contact. Alternatively, the results in this study may have been affected by the number of cats suffering from acute terminal disease that were not tested and the probability that severely ill stray cats may be less likely to be examined than similarly sick privately owned cats. In this case the results may underrepresent the prevalence of FeLV among stray cats.

Antibodies to FIV were recorded in 4.9 per cent of the healthy cats and 16.7 per cent of the sick cats, prevalences which were similar to those observed in privately owned cats (Hosie and others 1989, Shelton and others 1989, Ueland and Lutz 1992). FIV is thought to be spread predominantly by territorial aggression and biting (Yamamoto and others 1989), activities which are common among stray cats. Studies by Hosie and others (1989), Yamamoto and others (1989) and Ishida and others (1989) have recorded a higher prevalence of FIV in older, entire male cats, a trend which was also observed in this study. Age, sex and non-traumatic illness were shown to have independent associations with FIV antibody-positive status. Multivariate analysis indicated that feral status and body condition were not independently associated with FIV infection, suggesting that these two factors may have been associated with other factors that were themselves associated with infection, for example, perhaps FIV-infected cats were more likely to be ill and ill cats were more likely to be underweight.

There were small numbers of cats in several groups, especially FeLV-positive cats and pedigree cats, and statistically significant results were therefore difficult to establish from the analyses. However, there were some interesting trends with respect to the cats which were ill when they entered the hospital. Old or neglected injuries seemed to be associated with FIV, supporting perhaps the association of a wandering lifestyle with FIV status. The link between FIV and anaemia has been reported by Yamamoto and others (1989) and Hopper and others (1989). The possible link between FIV and renal disease agrees with the results of Thomas and others (1993) who found an association between FIV infection, small kidneys and azotaemia. Similarly, FeLV was associated with gingivitis and tumours, and FIV with severe oral disease and abscesses, associations which have all been frequently reported (Hardy 1980, Knowles and others 1989, Reinacher 1989, Yamamoto and others 1989, Friend and others 1990).

The prevalence of seropositivity to FCoV was 22.4 per cent, a similar prevalence to that previously recorded in the UK in both healthy and sick cats. The higher prevalence of FCoV antibodies in cats over two years old has also been recorded (Horzinek and Osterhaus 1979). It was surprising that FCoV antibodies were recorded more frequently in feral or semiferal cats than in tame cats, because the virus is shed in the faeces and is unlikely to survive for more than a few days outdoors, whereas in dried cat litter it may survive for a few weeks. As a result tame cats that share litter trays are thought more likely to become infected than feral cats that bury their faeces outdoors. Horzinek and Osterhaus (1979) have postulated that a cat's movement through several territories may lead to a higher risk of FCoV infection and feral cats may have more chance of using several territories as they avoid human contact.

Three of the FVU test results for FeLV were discordant (ELISA positive but virus isolation negative). These could have been

due to false ELISA results or they may have been true discordant results in which FeLV p27 antigen was present, but virus isolation was negative. This can occur very early in a FeLV infection, before the whole virus is being produced by the bone marrow and viraemia occurs. Cats with such discordant results are usually retested periodically until they become either positive or negative by both p27 antigen and virus isolation.

The results of the RIM tests correlated well with the results of the other tests. However, false negatives and false positives appeared to occur. When the FeLV or FIV test results do not agree with a cat's clinical picture or history, the cat should be retested with a different technology. After this study had been completed the manufacturers of the RIM test advised that all coloured ghost bands were to be considered positive and noncoloured ghost bands negative. The colour of the ghost bands was not recorded during the study.

Few of the stray cats were reclaimed, hence, most of them would have had to be dealt with by the authority accepting them. This indicates that many stray cats are likely to need to be rehomed and prevalence data on FIV and FeLV should be of value.

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#### References

- ADDIE, D. D. & JARRETT, O. (1992a) A study of naturally occurring feline coronavirus infections in kittens. *Veterinary Record* 130, 133-137
- ADDIE, D. D. & JARRETT, O. (1992b) Feline coronavirus antibodies in cats. Veterinary Record 131, 223-224
- FRIEND, S. C. E., BIRCH, C. J., LORDING, P. M., MARSHALL, J. A. & STUD-DERT, M. J. (1990) Feline immunodeficiency virus: prevalence, disease associations and isolation. Australian Veterinary Journal 67, 237-242
- GRUFFYDD-JONES, T. J., HOPPER, C. D., HARBOUR, D. A. & LUTZ, H. (1988) Serological evidence of feline immunodeficiency virus infection in UK cats from 1975-76. Veterinary Record 123, 569-571
- HARDY, W. D. Jr (1980) Feline Leukemia Virus. Amsterdam, Elsevier North Holland. pp 3-28
- HOPPER, C. D., SPARKES, A. H., GRUFFYDD-JONES, T. J., CRISPIN, S. M., MUIR, P., HARBOUR, D. A. & STOKES, C. R. (1989) Clinical and laboratory findings in cats infected with feline immunodeficiency virus. *Veterinary Record* 125, 341-346
- HORZINEK, M. C. & OSTERHAUS, A. D. M. E. (1979) Feline infectious peritonitis: A worldwide serosurvey. American Journal of Veterinary Research 40, 1487-1492
- HOSIE, M. J. & JARRETT, O. (1990) Serological responses of cats to feline immunodeficiency virus. *AIDS* **4**, 215-220
- HOSIE, M. J., ROBERTSON, C. & JARRETT, O. (1989) Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. *Veterinary Record* **128**, 293-297
- ISHIDA, T., WASHIZU, T., TORIYABE, K., MOTOYOSHI, S., TOMODA, I. & PEDERSEN, N. C. (1989) Feline immunodeficiency virus infection in cats of Japan. Journal of the American Veterinary Medical Association 194, 221-225
- JARRETT, O., GOLDER, M. C. & STEWART, M. F. (1982) Detection of transient and persistent feline leukaemia virus infections. *Veterinary Record* 110, 225-228
- KNOWLES, J. O., GASKELL, R. M., GASKELL, C. J., HARVEY, C. E. & LUTZ, H. (1989) Prevalence of feline calicivirus, feline leukaemia virus and anti-

bodies to FIV in cats with chronic stomatitis. Veterinary Record 124, 336-338 LUTZ, H., PEDERSEN, N. C., DURBIN, R. & THEILEN, G. H. (1983) Monoclonal antibodies to three epitopic regions of feline leukemia virus p27 and their use in enzyme-linked immunosorbent assay of p27. *Journal of Immunological Methods* 56, 209-220

- PEDERSEN, N. C., HO, E. W., BROWN, M. L. & YAMAMOTO, J. K. (1987) Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235, 790-793
- REINACHER, M. (1989) Diseases associated with spontaneous feline leukemia virus (FeLV) infection in cats. *Veterinary Immunology and Immunopathology* **21**, 85-95
- SHELTON, G. H., WALTIER, R. M., CONNOR, S. C. & GRANT, C. K. (1989) Prevalence of feline immunodeficiency virus and feline leukemia virus infections in pet cats. *Journal of the American Animal Hospital Association* 25, 7-12

SPARKES, A. H., GRUFFYDD-JONES, T. J., HOWARD, P. E. & HARBOUR,

D. A. (1992) Coronavirus serology in healthy pedigree cats. *Veterinary Record* 131, 35-36

- THOMAS, J. B., ROBINSON, W. F., CHADWICK, B. J., ROBERTSON, I. D. & BEETSON, S. A. (1993) Association of renal disease indicators with feline immunodeficiency virus infection. *Journal of the American Animal Hospital* Association 29, 320-326
- UELAND, K. & LUTZ, H. (1992) Prevalence of feline leukemia virus and antibodies to feline immunodeficiency virus in cats in Norway. *Journal of Veterinary Medicine* 39, 53-58
- YAMAMOTO, J. K., HANSEN, H., HO, E. W., MORISHITA, T. Y., OKUDA, T., SAWA, T. R., NAKAMURA, R. M. & PEDERSEN, N. C. (1989) Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. Journal of the American Veterinary Medical Association 194, 213-220

# Evaluation of the portable Cepheid SmartCycler real-time PCR machine for the rapid diagnosis of foot-and-mouth disease

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The ability of the portable Cepheid SmartCycler real-time PCR machine to detect foot-and-mouth disease (FMD) virus sensitively and accurately was evaluated by comparing the results of the analyses of nasal swab and serum samples from experimentally infected animals with those obtained from the real-time PCR assay currently in use in the laboratory. The results indicated that the ability of the machine to detect viral RNA is greatly affected by the PCR reagents used for the assay. When it was used with PCR beads it was unable to detect weakly positive samples, but when TaqMan core reagents were used for the assay, its sensitivity was significantly increased. The machine could be used for the laboratory-based detection of FMD; however, as with all assays, significant optimisation of assay conditions as well as solid validation of the technique is required.

THE rapid reporting and diagnosis of foot-and-mouth disease (FMD) and the rapid implementation of diseasecontainment measures are fundamental for the control and eradication of the disease. Its clinical diagnosis can be more difficult when sheep are affected because in this species the disease often produces only mild and transitory clinical signs which are difficult to identify (Callens and others 1998). In suspected cases of FMD in sheep, there is therefore likely to be even greater reliance on laboratory confirmation, which must be rapid so that control measures can be introduced without delay.

Samples submitted to the OIE/FAO World Reference Laboratory at Pirbright for the investigation of FMD virus include vesicular epithelium from lesions, blood or serum, swab samples and occasionally milk. Vesicular epithelium, particularly from the foot, is the most valuable sample, owing to its high viral content during the early acute phase of the disease (Oliver and others 1988). At present, the OIE-approved test for FMD diagnosis is the antigen-detection ELISA (OIE 2000), which can detect a positive specimen in three to four hours (Ferris and Dawson 1988). However, a negative ELISA result may be obtained when a specimen is from an old lesion, or is small, or contains too little virus to be detected. The method used to determine whether any infectious virus is present is to attempt to isolate the virus by inoculating and passaging the original tissue suspension through susceptible cell cultures, and looking for a cytopathic effect (cpe). Any sample suspected of showing a cpe is subjected to an ELISA to determine the presence of FMD virus. Each passage in cell culture takes 48 hours, and it may therefore take several days to confirm a weak positive sample, a delay which may compromise control measures in the field. An additional limitation of the ELISA method is that it is unsuitable for detecting FMD virus in specimens of blood or serum, swab samples, or milk, so these samples are generally tested for virus by direct inoculation into cell culture, and subsequent ELISA testing of any cultures showing a cpe.

A conventional PCR has been assessed as a diagnostic replacement for the ELISA but its value is limited owing to the small number of samples that can be tested simultaneously and by its relative insensitivity (Reid and others 1998). The fluorogenic real-time PCR, which had been shown to be very sensitive for the detection of FMD virus in experimental animals (Alexandersen and others 2001, 2002, Oleksiewicz and others 2001), was therefore investigated as a possible diagnostic tool. A slightly modified 5'nuclease probe-based fluorogenic PCR assay was developed which has recently been shown to detect FMD viral RNA accurately in a wide range of tissue samples as sensitively as viral isolation, and more sensitively than ELISA (Reid and others 2001). The introduction on to the market of portable, real-time PCR machines, such as the Cepheid SmartCycler, raised the question whether such a machine could be used for the diagnosis of FMD in the field, thus avoiding the need for the often time-consuming packaging and transportation of samples to a central testing laboratory. The suitability of the SmartCycler PCR machine for the rapid and accurate detection of FMD virus was therefore

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# Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus and feline coronavirus in stray cats sent to an RSPCA hospital

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