



Isolation of feline herpesvirus-1 and feline calicivirus from healthy cats in Swedish breeding catteries

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Feline calicivirus (FCV) could be isolated from four cats (2.6%) and feline herpesvirus-1 (FHV) from none of 152 clinically healthy cats from 22 Swedish breeding catteries. These cats had all previously shown signs of respiratory tract disease or conjunctivitis, although several years ago. The results suggest that carriers of FCV and FHV were uncommon in Swedish breeding catteries studied. Prevalence rates in other European countries and North America are usually higher, especially of FCV. The lower prevalence rates in our study might be explained by test group selection, differences in factors such as management, environment, or genetic constitution of the cats, or by sample handling. It was concluded that the presence of an FCV shedder in the cattery does not mean that all cats in the group are infected, but special measures are recommended to avoid infection of susceptible cats.

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Despite the widespread use of vaccines against feline herpesvirus-1 (FHV, feline rhinotracheitis virus) and feline calicivirus (FCV) in breeding catteries, infections with these viruses are still common, especially when cats are kept in groups (Dawson et al 2001). One possible reason for this is that even if cats are vaccinated against FCV and FHV, they can become virus carriers if challenged (Orr et al 1978, Gaskell et al 1982, Pedersen and Floyd Hawkins 1995), and infect susceptible in-contact animals (Gaskell et al 1982). Another reason might be that kittens of persistently infected queens can get infected and experience sub-clinical or mild infection before vaccination, when the maternally derived antibodies are lowered at 3–9 weeks of age (Johnson and Povey 1984, Yamada et al 1991, Casal et al 1996). Queens chronically infected with FCV shed virus constantly. The stress caused by lactation may lead to reactivation of a latent FHV infection in queens, leading to virus shedding (Gaskell and Povey 1977) and possible transmission.

Clinical disease caused by the two viruses is commonly most severe in young animals. Signs caused by FHV are usually confined to the respiratory tract, although abortions have been induced experimentally (Hoover and Griesemer 1971). Clinical signs include depression, marked sneezing, hypersalivation, conjunctivitis, and oculonasal discharges. Pneumonia and high mortality might be seen in young kittens (Gaskell and Dawson 1998). Clinical disease caused by FCV is typically characterised by oral ulcerations with or without mild respiratory and conjunctival signs (Gaskell and Dawson 1998). A transient and shifting lameness is not unusual (Pedersen et al 1983), and some authors consider it the most common clinical presentation (Pedersen 1991). Strains of FCV differ in antigenicity and virulence, and the virus has also been associated with a haemorrhagic-like fever (Pedersen et al 2000, Schorr-Evans et al 2003), abortion (Ellis 1981a, van Vuuren et al 1999) and chronic stomatitis (Knowles et al 1989).

In the year 2000, 5% of the samples for virus isolation that were sent to the Department of Virology at the National Veterinary Institute were positive for FHV and 18% were positive for FCV (unpublished data). A higher prevalence

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of FCV than that of FHV in cats with respiratory disease has been reported in several studies (Harbour et al 1991, Binns et al 2000, Mochizuki et al 2000, Cai et al 2002), but the opposite situation has also been described (Sykes et al 2001).

The prevalence of healthy, FCV-positive animals has been described to vary between 15% and 25% (Ellis 1981b, Harbour et al 1991, Coutts et al 1994) and healthy FHV-positive cats between 0.2% and 33.3% (Ellis 1981b, Harbour et al 1991, Coutts et al 1994, Stiles et al 1997a,b, Maggs et al 1999). The proportion of healthy cats in Swedish breeding catteries that excrete FCV and FHV has hitherto not been studied.

The aims of this study were to evaluate the prevalence of FCV and FHV positive clinically healthy cats in a small number of Swedish breeding catteries, as determined by virus isolation after a single sampling, and to see if there was any correlation between virus positive cats and management, such as number of cats in the cattery.

Materials and methods

Cats

A total of 152 clinically healthy cats from 22 breeders were included in the study. Breeds included were Abyssinians, Birman, Burmese, Cornish Rex, Devon Rex, Exotic Shorthair, Norwegian Forest cats, Persian, Siamese, and Oriental Shorthair, as well as domestic shorthair and domestic longhair cats that were kept together with the pedigree cats. There were six breeders of shorthair breeds with a mean of 5.8 adult cats per breeder, 12 breeders of semi-longhair breeds with a mean of 4.7 adult cats per breeder and four breeders of Persians and Exotic Shorthair with a mean of 10.2 adult cats per breeder.

The cats were sampled by one of the authors (BSH) in the catteries, and for each cat data were recorded on age, previous matings, if the cats had visited any cat show, if they had experienced periods with conjunctivitis or upper respiratory tract disease, lameness as kittens, gingivitis or any reproductive disturbances and if they were born in a cattery with more than six adult cats.

Sampling methods and virus isolation

Oropharyngeal and conjunctival swabs were collected and placed in virus transport medium

(Virocult; Medical Wire & Equipment Co, England) and transported to the laboratory within 24 h. At the laboratory, the swabs were placed in 12 ml tubes containing 1 ml PBS with 75 µl penicillin/streptomycin and 50 µl fungizone and put in to a -70°C deep freezer. The tubes were thawed at room temperature immediately before inoculation of cell cultures. From 18 breeders (130 cats) the ocular and oropharyngeal swabs from each individual cat were pooled before the virus isolation procedure, whereas from four breeders (22 cats) all swabs were treated individually. After low-speed centrifugation, 200 µl of each supernatant were inoculated into 10 ml duplicate tissue culture tubes containing subconfluent monolayers of *Felis catus* whole fetus (fcwf) cells and incubated at 37°C (Vennema et al 1991). Cell cultures were observed daily for cytopathic effects (CPE), and cultures showing CPE were investigated for the presence of herpesvirus or calicivirus by electron microscopy (Englund et al 2002). Cultures not showing CPE were blind passaged twice. If no CPE was detected, the cultures were considered negative for virus isolation.

Statistical analysis

Comparison of prevalence of virus positive cats in the shorthair, longhair and semi-longhair catteries were performed using Fisher's exact test and calculation by hand, otherwise ANOVA or Student's *t*-test and the Minitab statistical package (Minitab release 13, Minitab Inc., State College, PA) were used. Differences were considered significant when $P < 0.05$.

Results

The mean age of the cats that were sampled was 4 years: 5.4 years for the shorthair group (range 0.23–14 years), 3.9 years for the semi-longhair group (range 0.15–13 years) and 2.8 years for the group of Persians and Exotic Shorthairs (range 0.25–8 years). Of the cats that were sampled, 60% were born in catteries with more than six adult cats. Eighty-four percent of the 152 cats had visited cat shows and 66% had mated; 17% had mated or visited a show 3 weeks or less before sampling (Table 1). All cats more than 10 weeks old were vaccinated against FCV and FHV, and adult cats had been given regular, usually annual, boosters. Forty-eight cats (32%) had been vaccinated with an attenuated live vaccine,

Table 1. General description of cats included in the study

	All cats	Shorthair	Semi-longhair	Persian/Exotic Shorthair
Number of cats	152	43	62	47
Number of breeders	22	6	12	4
Age (mean and range, years)	4.0 (0.15–14)	5.4 (0.23–14)	3.9 (0.15–13)	2.8 (0.25–8)
% Female cats	71.1	79.1	67.7	68.1
% Neutered cats	23.7	32.6	22.6	17.0
% Cats bred in cattery with >6 adult cats	60.5	46.5	50.0	87.2
% Cats with mating experience	66.4	62.8	67.7	68.1
% Cats that has visited shows	83.6	93.0	79.0	80.9
% Cats that had mated or visited a show within 3 weeks before sampling	17.1	18.6	8.1	27.7

78 (51%) with an inactivated vaccine and 26 kittens (17%) had not yet been vaccinated.

Of the 22 breeders, 15 (68%) had previously observed cats with conjunctivitis in the cattery and 10 (45%) had experienced cats with rhinitis (Table 2). Fifteen breeders (68%) had experienced problems with reproductive disturbances in the cattery. Of the 152 cats sampled, 21% had previously shown signs of conjunctivitis and 18% of rhinitis. Two percent of the cats had been lame as kittens, and 0.7% had shown signs of gingivitis (Table 2). Twenty-two percent had experienced some kind of reproductive disturbances (small litters, stillborn kittens, infertility, or dystocia), but no case of abortion was reported.

Feline herpesvirus was not isolated from any of the cats. Feline calicivirus was isolated and confirmed by electron microscopy from four cats (2.6%), two of which lived in the same cattery, and was thus detected in three (14%) of the catteries. Virus was detected from the pools of ocular and oropharyngeal swabs. None of the virus positive cats had experienced any period of lameness as kittens, they had not had any reproductive problems except for one that had experienced dystocia caused by malpresentation

of a fetus, and they showed no sign of gingivitis. All had been regularly vaccinated with an inactivated vaccine. All four cats belonged to the shorthair breeds (Table 3).

Thus, FCV was isolated in three out of six catteries with shorthair cats (excluding Exotic Shorthair) and the mean age of cats that were FCV positive was 10 years. FCV was not isolated from any semi-longhair cat or from any Persian or Exotic Shorthair. The difference in prevalence of FCV-positive cats between catteries with shorthair cats and catteries with semi-longhair and longhair cats (grouped together) was significant, as was the difference in prevalence between shorthair catteries and semi-longhair catteries, whereas the difference between shorthair and longhair catteries was non-significant. The prevalence of FCV did not vary significantly depending on the age of the cat, number of cats in the cattery or whether the cat was born in a cattery with more than six cats or not.

Discussion

The present study differs from previous studies on healthy virus carriers in that all cats in a small number of well-described catteries were

Table 2. Previous history of clinical signs that might be associated with FCV or FHV infection

Previous history of:	All breeds		Shorthair breeds except Exotic Shorthair		Semi-longhair breeds		Persians and Exotic Shorthair	
	% Breeders	% Cats	% Breeders	% Cats	% Breeders	% Cats	% Breeders	% Cats
Conjunctivitis	68.2	17.8	50	18.6	67	12.9	100	34.0
Rhinitis	45.5	21.1	67	20.9	25	12.9	75	21.8
Lame as kittens	13.6	2.0	0	0	25	4.8	0	0
Gingivitis	4.5	0.66	0	0	8.3	1.6	0	0

Table 3. Characteristics of FCV-positive cats

Cattery	Number of cats in cattery	Breed	Age (years)	Gender	Born in a cattery with more than 6 cats	Previous signs of URTD	Time since last mating	Time since last visit to cat show
A	10	Cornish Rex	15	F	Yes	5 years ago	>1 year	>1 year
A	10	Siamese	9	F	Yes	Occasional sneezing	>1 year	>1 year
B	4	Burmese	13	NF	Yes	4 years ago	>1 year	>1 year
C	7	Oriental Shorthair	4	F	Yes	Conjunctivitis as kitten	10 days	5 months

yrs = years, URTD = upper respiratory tract disease, F = female, NF = neutered female.

sampled. In other studies, it has been common to study a single or just a few cats per cattery. Anamnestic data regarding previous respiratory tract disease and other clinical signs that might be related to infection with FCV or FHV were recorded for each cat.

Feline herpesvirus-1 was not isolated from any cat in this study. Previous studies also report low isolation rates of FHV from clinically healthy cats. In a study on clinically healthy cats at cat shows, FHV was isolated from only 0.58% (Coutts et al 1994). Wardley et al (1974) isolated FHV from 1% of clinically healthy household pet cats, 1.75% of cats at shows and 1.75% of colony cats. In a more recent investigation, 1% of clinically healthy cats were positive for FHV, with no association between the number of cats in the household, or the type of household, and isolation of FHV (Binns et al 2000). There might be regional differences in the prevalence of FHV, as 10.9% of clinically normal cats in North America were positive for FHV by virus isolation (Maggs et al 1999).

There are several possible reasons why no FHV-positive cat was identified. First, it might be because no cat was infected. Although 68.2% of the breeders reported a history of conjunctivitis in their cattery, no cat showed clinical signs when the samples were taken. It is possible that other infectious agents, such as *Chlamydomphila felis* or *Mycoplasma felis* caused the previous clinical signs. It is also possible that there were latent carriers that were not shedding virus at the time of sampling. Six months after infection with FHV, only 4% (20/524) of the cats spontaneously shed virus (Reubel et al 1993). The carrier state of FHV is characterised by a latent phase with shorter periods of shedding, often preceded by a stressful situation. Virus shedding usually starts around 1 week after a stressful situation

and continues for 1–2 weeks (Gaskell and Povey 1973, 1977). Only 26 cats in the present study (17.1%) had visited a cat show or mated (both can be stressful situations) within 3 weeks before sampling, and thus were of greater risk of stress-related shedding of FHV, should they be carriers.

The cats sampled might also have been low-grade shedders and virus isolation not a method sensitive enough. With a more sensitive method, such as nested PCR, some FHV infected cats might have been detected (Reubel et al 1993, Stiles et al 1997b).

FCV is isolated more commonly than FHV from cats with upper respiratory tract disease (Harbour et al 1991, Binns et al 2000, Mochizuki et al 2000, Cai et al 2002). However, whereas FHV-positive cats probably are infected for their lifetime, many cats stop shedding FCV within a month (Knowles et al 1991, Truyen et al 1999) although some become chronic carriers (Wardley and Povey 1976). Persistent infection with FCV is characterised by continuous shedding (Wardley and Povey 1976), although negative culture results occur (Hurley and Sykes 2003). The proportion of chronically infected cats from which FCV can be detected can thus be expected to be higher than the proportion of cats chronically infected with FHV that are positive on virus isolation.

For FCV, the isolation rates in an early study were shown to be higher from cats sampled at cat shows than from household pets, and very high in some cat colonies (Wardley et al 1974). Isolation rates have also been shown to be higher in young (<1 year old) than in older animals (Wardley et al 1974, Coutts et al 1994). In a more recent study, there was no association between FCV isolation and age of the cat, the number of cats in the household or with the household type, respectively, whereas positive correlations were

observed between FCV isolation and respiratory disease, recent antibiotic therapy, and contact with dogs (Binns et al 2000). None of the four FCV-positive cats in the present study was less than 12 months old and their mean age was 10 years. They came from small or medium sized catteries, and three of them had neither visited a cat show nor mated recently. Common characteristics were that they were all bred in catteries with more than six adult cats, they were all females (intact or neutered), shorthair or oriental breed and they had all previously experienced some clinical signs of upper respiratory tract disease (one of them with only the eyes involved), although several years ago. It is, therefore, possible that they were chronic carriers. The fact that they were all shorthair or oriental breed might implicate a breed predisposition. Coutts et al (1994) found a significant difference between breeds in their study, with the highest prevalence of FCV-positive cats in the longhair breeds and the lowest in household pets. Higher prevalence within certain breeds may point to an increased susceptibility for infection, differences in environment and management, or simply the fact that once introduced within a breed, virus spread occurs more efficiently within the breed due to closer contact between cattery cats of the same breed.

Virus isolation is the most reliable microbiological assay for FCV (Hurley and Sykes 2003). It has previously been suggested that storage at -70°C reduces the chances of detecting FCV (Komolafe 1979, Sykes et al 2001), although in the study by Komolafe (1979) purified feline calicivirus particles were stored, that are likely to be more sensitive than when different proteins are present, such as in clinical samples. In many diagnostic laboratories, including the one used in the present study, samples are routinely frozen before virus isolation without any observed negative effects on the detection rates. The low isolation rates are not likely to be caused by the pooling of swabs, as all positive cats were detected in pooled samples. We do, therefore, believe that the low isolation rate is a true reflection of a low proportion of virus shedders. The relatively low prevalence of FCV-positive cats in the present study might be explained by genetic or management factors in the studied catteries. Previous studies describe prevalence in other populations, such as cats attending cat shows (Coutts et al 1994), cat refuge homes (Ellis 1981b) or samples from veterinary practices (Harbour et al 1991).

Vaccination against FCV usually reduces clinical signs (Pedersen and Floyd Hawkins 1995) but does not eliminate clinical illness due to FCV, and a large proportion of cats with upper respiratory tract disease from which FCV can be isolated have been vaccinated (Harbour et al 1991). Furthermore, vaccination does not protect against the chronic carrier state (Gaskell et al 1982, Pedersen and Floyd Hawkins 1995). Evidence has even been presented suggesting that the endemic virus strain in an infected cat colony can evolve from the virus strain in a live, attenuated vaccine (Radford et al 2001b).

As the FCV-positive cats in the present study did not show signs of clinical illness, and as all cats in the breeding catteries were regularly vaccinated, the greatest risk for the breeders with these cats would be infection of small, not yet vaccinated kittens leading to possibly severe clinical signs. Booster vaccination of queens in connection with mating is recommended to ensure high levels of maternal antibodies in the colostrum (Ström Holst 2002). Queens with kittens should also be kept separately from other cats, to reduce the risk of other cats in the cattery infecting the kittens. It is clear from the present study, that in a group of cats, the majority can be virus negative, even when there is a carrier within the group. Low evidence of virus transmission within a cat rescue shelter has previously been reported by Radford et al (2001a). In breeding catteries where respiratory tract disease is a problem, kittens can be vaccinated from 6 weeks of age (Dawson et al 2001).

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