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# SHORT COMMUNICATION
# Feline herpesvirus 1 and feline calicivirus infections in a heterogeneous cat population of a rescue shelter

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Feline herpesvirus 1 (FeHV-1) and feline calicivirus (FCV), associated with upper respiratory tract disease, are highly prevalent in cats worldwide. With the aim to investigate the importance of feline respiratory viruses in a heterogeneous population of cats, samples were taken in a rescue shelter in Liège, Belgium, between March 2005 and August 2006. Reverse transcription polymerase chain reaction (RT-PCR) and polymerase chain reaction (PCR) were performed to diagnose FCV and FeHV-1 infection in the sampled cats. The prevalence rate (33.1%) was higher for FCV than for FeHV-1 (20.1%) whereas prevalence rate of co-infection with both viruses was 10%. Gingivitis was more common in FCV infections (odds ratio (OR) = 2.83) whereas respiratory signs were more often observed with FeHV-1 infections. The average age was significantly higher in FCV positive cats (38 months) than in FeHV-1 positive cats (29.9 months). The second and the fourth quarters of the year and the two first quarters were significantly more at risk than the others in the case of FeHV-1 and FCV infection, respectively. Age was found to be a confounding factor. High prevalence of both infections strengthens the importance of applying hygienic and preventive measures in rescue shelters where cats with an unknown status of vaccination are introduced.

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Feline herpesvirus 1 (FeHV-1) and feline calicivirus (FCV) are the main agents involved in the feline upper respiratory tract disease (URTD).[1,2] These viruses are responsible for acute illness and may also be the cause of recurrent or chronic lesions.

FeHV-1 is a double-stranded DNA virus, member of the *Varicellovirus* genus of the subfamily *Alphaherpesvirinae*.[3,4] It has a tropism for nasal epithelial and conjunctival cells and for neurocytes.[5] Trigeminal ganglia are the site of FeHV-1 latent infection[6] and viral reactivation can occur during a stress period.[7]

FCV is a single-stranded positive-sense RNA virus in the family *Caliciviridae*, genus *Vesivirus*. There is considerable genetic diversity within FCV; within a strain, genetic variation tends to be higher in endemically infected cat populations.[8,9] The most likely hypothesis is that circulating virus is subjected to a positive selection pressure induced by the immune response of infected cats, leading to virus evolution and the generation of new strains.[9] Furthermore, such evolution may also in part explain vaccine protection failures against wild-type FCV.[9,10]

The aim of this longitudinal study was to gain a better knowledge of the epidemiology of feline respiratory viruses in a heterogeneous population of cats in a rescue shelter that experiences a continuous turnover of animals. Oral swabs and polymerase chain reaction (PCR) analyses allowed the study of FeHV-1 and FCV prevalence, and also clinical signs caused by single or mixed infections. Viral infections were investigated irrespectively of the clinical status of the animal. The investigations analysed the effect of several risk factors, ie, sex, age and season, on FCV and FeHV-1 infections. For each cat, age, sex, vaccine used at his arrival and clinical signs were recorded by the same two veterinarians: presence or absence of gingivitis, salivation, oral ulcers, lacrymation, conjunctivitis, nasal discharge, sneezing, stertor or cough.

A total of 299 cats from the Société Royale Protectrice des Animaux (SRPA), Liège, were included in the study. The sample size was calculated according to a population size of 1500 cats (average number of cats entering SRPA during 1 year), an expected

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prevalence of 40%[2] and an accepted error of 5% with a confidence interval (CI) of 95%. Average age of the sampled cats was 34 months (2−180). An average of 21 cats (from 16 to 26) in the quarantine area was sampled (one selected randomly in order of appearance when entering each cage) every month, between March 2005 and August 2006. Each cat was sampled once. The quarantine area was used for putting in new arrivals, in case they were incubating something.

Oropharyngeal swabs were collected and placed in 2 ml of culture medium (Gibco MEM supplemented with antibiotics) and stored in the laboratory at 4°C. Each sample was inoculated on confluent monolayers of susceptible Crandell Rees feline kidney (CrFK) cells and incubated at 37°C in a moist atmosphere with 5% $CO_2$ for 3 days and examined for characteristic cytopathic effects (CPEs).

Viral nucleic (RNA and DNA) acid was extracted from the supernatant of each inoculated monolayer by using QIAamp viral RNA mini spin protocol (Qiagen, Hilden, Germany).

Two sets of primers, with a concentration of 400 nM, for calicivirus detection were used. A conventional reverse transcription polymerase chain reaction (RT-PCR) for polymerase gene detection of FCV using the set of primers p30F/p30R was performed to amplify a 126 base pair (bp) sequence of FCV p30 gene.[11] The primer pair V2F/V2R was used to amplify a 700 bp sequence of the FCV capsid protein VP1gene.[12] The Access RT-PCR System kit from Promega was used with 3 µl of RNA template and according to manufacturer's instructions. Annealing in the RT-PCR cycle was performed at 60°C for p30 primer pairs and at 61°C for V2 primer pairs for 30 s. An amplification with one of the two primers pairs indicated that the sample was positive for FCV.

The set of primers gC-R/gC-F, with a concentration of 100 nM, was used to diagnose FeHV-1 by the amplification of a 500 bp sequence located in the gene coding for glycoprotein gC.[13] PCR was carried out using 2−5 units of *Taq* DNA polymerase (Biolabs, Leusden, The Netherlands) and 5 µl of DNA template, annealing performed at 59°C for 1 min.

Negative controls were performed in each test by adding water and supernatant of uninfected CrFK cells in the place of the nucleic acid template. PCR positive controls were performed with vaccine strains. For FeHV-1 detection, 5 µl of the control were used (75.11 ng/µl), whereas for FCV detection, only 3 µl of the control were used (62.72 ng/µl). PCR product sizes were determined by agarose gel electrophoresis and ethidium bromide staining.

The infection prevalence of FCV and FeHV-1 was estimated using a binomial exact distribution. The mean age difference of infected cats with FCV or FeHV-1 was assessed with the Welch's test ($P \leq 0.05$).[14] Sex and quarter of year effects, and relation between clinical signs observed and virus infection were analysed using a $\chi^2$ test and by mean of odds ratios (ORs) with 95% CI; a $P$ value $< 0.05$ was considered as significant.[15] The possible relationship between the infection level with FCV or FeHV-1 in cats for different quarters of the year was assessed by a Cochran−Mantel−Haenszel (CMH) method ($OR_{MH}$). The Breslow−Day test was used to evaluate the homogeneity of the ORs.

The prevalence rate of FCV alone, 33.1% (99/299) (95% CI: 27.8−38.8%), during the observation period, was higher than the FeHV-1, without FCV infection, prevalence rate, 20.1% (60/299) (95% CI: 15.7−25.1%), and than the prevalence of co-infection with both viruses, 10% (30/299) (95% CI: 6.8−14%). Table 1 shows that the use of two FCV primer pairs was appropriate as some samples were positive for FCV only with one of the two primers sets. Furthermore, even without obtaining a CPE, some samples were found positive by RT-PCR on the infected cell culture supernatant (Table 1).

Among the 67 samples that reacted negative to FCV RT-PCR but showed a CPE, 49 were found FeHV-1 positive (Table 1). The remaining 18 samples which appeared to show CPE but were negative by PCR for both viruses may have been contaminated with fungi or bacteria at sampling time or alternatively the apparent CPE was due to possible toxic effects of the samples on the cells: further cell passage of the inoculated cultures may have clarified this. It is also possible that there may have been sensitivity issues with the PCRs used, particularly for FCV. Indeed the FCV RT-PCR performed with the conserved polymerase p30 gene[11] was more sensitive (116 positives detected) than that performed on the hypervariable region of the capsid protein gene[12] (79 positives detected) (Table 1). Thus using both primer pairs for FCV clearly helped maximise the overall sensitivity of detection, but because of the

**Table 1**. RT-PCR results with the two primer pairs for FCV detection in infected cell culture supernatant (p30: RT-PCR with p30 primer pair; V2: RT-PCR with V2 primer pair) compared with the FeHV-1 PCR results

| CPE | FCV RT-PCR | | | FeHV-1 PCR positive samples |
|---|---|---|---|---|
| | p30 | V2 | *N* | *N* |
| + | + | + | 55 | 10 |
| | + | − | 35 | 14 |
| | − | + | 9 | 3 |
| | − | − | 67 | 49 |
| − | + | + | 11 | 0 |
| | + | − | 15 | 3 |
| | − | + | 4 | 0 |
| | − | − | 103 | 11 |

CPE = cytopathic effect; FCV = feline calicivirus; RT-PCR = reverse transcription polymerase chain reaction; FeHV-1 = feline herpesvirus 1; PCR = polymerase chain reaction; *N* = Number of animals.

**Table 2**. Adjusted ORs calculated for selected clinical signs between four groups of animals (FCV group ($n = 99$), FeHV-1 group ($n = 60$), FCV and FeHV-1 group ($n = 30$) and group without viral infection ($n = 110$))

| Clinical signs and lesions | Number of animals by group | | | | OR (95% CI)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | FCV, *n* | FeHV-1, *n* | FCV & FeHV-1, *n* | WVI, *n* | FCV versus WVI | FeHV-1 versus FCV | FCV & FeHV-1 versus FCV | FeHV-1 versus WVI | FeHV-1 versus FCV & FeHV-1 | FCV & FeHV-1 versus WVI |
| Gingivitis | 41 | 19 | 10 | 22 | 2.83(1.53−5.23) + | 0.66(0.33−1.29) | 0.71(0.30−1.67) | 1.85(0.90−3.80) | 0.93(0.36−2.36) | 2.00(0.82−4.88) |
| Salivation | 1 | 4 | 2 | 0 | 3.37(0.14−83.57) | 7.00(0.76−64.18) | 7.00(0.61−80.07) | 17.6 (0.93−332.73) | 1.00(0.17−5.79) | 19.39(0.91−415.20) |
| Oral ulcers | 5 | 3 | 1 | 2 | 2.87(0.54−15.15) | 0.99(0.23−4.30) | 0.65(0.07−5.78) | 2.84(0.46−17.50) | 1.53(0.15−15.33) | 1.86(0.16−21.26) |
| Other oral lesions | 0 | 0 | 0 | 3 | 0.15(0.01−3.03) | 1.64(0.03−83.97) | 3.26(0.06−167.90) | 0.25 (0.01−5.00) | 0.50(0.01−26.03) | 0.50(0.03−10.02) |
| Lacrymation | 7 | 8 | 6 | 4 | 2.02(0.57−7.11) | 2.02(0.69−5.89) | 3.29(1.01−10.69) + | 4.08(1.17−14.16) + | 0.62(0.19−1.97) | 6.63(1.73−25.31) + |
| Conjunctivitis | 3 | 3 | 0 | 5 | 0.66(0.15−2.82) | 1.68(0.33−8.63) | 0.45(0.02−8.90) | 1.11(0.25−4.79) | 3.71(0.19−74.25) | 0.31(0.02−5.85) |
| Nasal discharge | 11 | 19 | 10 | 8 | 1.59(0.61−4.14) | 3.71(1.62−8.50) + | 4.00(1.49−10.71) + | 5.91(2.40−14.56) + | 0.93(0.36−2.36) | 6.38(2.24−18.14) + |
| Sneezing | 12 | 30 | 17 | 11 | 1.24(0.52−2.96) | 7.25(3.30−15.94)+ | 9.48(3.70−24.30) + | 9.00(4.03−20.08) + | 0.76(0.32−1.85) | 11.77(4.53−30.54) + |
| Stertor | 4 | 8 | 3 | 4 | 1.12(0.27−4.59) | 3.65(1.05−12.71)+ | 2.64(0.55−12.52) | 4.08(1.17−14.16) + | 1.38(0.34−5.65) | 2.94(0.62−13.95) |
| Cough | 0 | 0 | 0 | 1 | 0.37(0.01−9.11) | 1.64(0.03−83.97) | 3.26(0.06−167.9) | 0.60(0.02−15.04) | 0.50(0.01−26.03) | 1.20(0.05−30.12) |

WVI = without viral infection; $n$ = number of animals with clinical signs; +: Risk factor statistically significant at $P \leq 0.05$.
*OR was adjusted when values of zero caused problems for OR calculations (adjustment of Grenier).

high sequence variability seen in FCV there may still have been other strains which were not detectable with the primer pairs used.

$\chi^2$ tests showed no significant difference ($P > 0.05$) between sex and origin of infection. On the other hand, Welch's test demonstrated that the average age of FCV positive cats was 38 months, which was significantly higher than the average age of FeHV-1 positive cats (29.9 months; $P \leq 0.05$). It was also higher than the average age of uninfected cats (32 months). In breeding catteries, FeHV-1 and FCV isolation in cats of 4−11 months old were positively associated with respiratory tract disease.[16] This difference between the investigated rescue shelter and breeding catteries can be explained by stressful conditions and high turnover in the cat population.

In the second and the fourth quarters of the year, cats were significantly more frequently infected by FeHV-1 than the two other quarters ($\chi^2 = 7.96$, $P = 0.005$) (OR = 2.07 (95% CI: 1.24−3.43)). The possible relationship between age and season was assessed for FeHV-1, by a CMH test ($OR_{MH} = 2.01$ (95% CI: 1.23−3.35)). The age of animals was found to be a confounding factor for the season because the crude OR was higher than the OR calculated for each age stratum: less than 1 year old (OR: 2.60 (95% CI: 0.90−7.47)), 1 year old (OR: 1.00 (95% CI: 0.26−3.93)), 2 years old (OR: 2.50 (95% CI: 0.71−8.80)), 3 years old (OR: 5.27 (95% CI: 1.59−17.5)), and more than 3 years (OR: 1.04 (95% CI: 0.37−2.88)). The Breslow test showed that the conditional ORs were homogeneously distributed ($P = 0.30$).

In the two first quarters, cats were significantly more frequently infected by the FCV than in the two later quarters ($\chi^2 = 11.4$, $P = 0.001$) (OR = 2.28 (95% CI: 1.41−3.69)). The possible relationship between age and season was assessed for FCV, by a CMH test ($OR_{MH} = 2.33$ (95% CI: 1.44−3.80)). The age of animals was found to be a confounding factor for the season because the crude OR was higher than the OR calculated for each age stratum: less than 1 year old (OR: 1.18 (95% CI: 0.41−3.37)), 1 year old (OR: 6.13 (95% CI: 1.95−19.26)), 2 years old (OR: 8.00 (95% CI: 1.45−44.30)), 3 years old (OR: 1.68 (95% CI: 0.48−5.85)), and more than 3 years (OR: 1.59 (95% CI: 0.65−3.88)). The Breslow test showed that the conditional ORs were homogeneously distributed ($P = 0.12$).

Adjusted OR revealed a relationship between virus infection and selected clinical signs (Table 2). However, 31.2% of sampled cats did not show any clinical sign in spite of positive PCR diagnosis for FCV or FeHV-1 (59/189). In cases of FCV positive cats exhibiting clinical signs, gingivitis was dominant (OR = 2.83, CI: 1.53−5.23). Respiratory signs like nasal discharge (OR = 3.71, CI: 1.62−8.50; OR = 5.91, CI: 2.40−14.56), sneezing (OR = 7.25, CI: 3.30−15.94; OR = 9.00, CI: 4.03−20.08), stertor (OR = 3.65, CI: 1.05−12.71; OR = 4.08, CI: 1.17−14.16) and lacrymation (OR = 4.08, CI: 1.17−14.16) were mainly reported in FeHV-1 infected cats (Table 2).

An age effect was demonstrated in cats infected by FeHV-1 and FCV, especially for FeHV-1 that infects younger cats.[16−18] These results could be explained by the protection of kittens by maternally derived antibodies. Furthermore, an immune-mediated mechanism for the acquisition of resistance to infection has been postulated in older cats.[19] FeHV-1 is rarely isolated from clinically healthy animals[20] because latent carriers are not expected to shed the virus at the precise time that they are sampled,[21] whereas FCV is continuously shed by infected cats.[22] In the investigated shelter, vaccination coincides with the arrival of new cats. However, vaccination is known to reduce but not prevent viral excretion[4,23] and thus cats already infected on arrival or subsequently infected might still shed either FCV or FeHV-1.

The increase in FeHV-1 prevalence observed in spring could be linked to the parturition period in the northern hemisphere. Some sampled cats are probably latent carriers of FeHV-1, but virus detection in swabs is only possible during reactivation−shedding period.[2] The true prevalence rate of FeHV-1 infection in the cat population from the SRPA in Liège is likely to be higher. The age of animals was found to be a confounding factor for the season. However, interactions with other factors cannot be definitively excluded. Therefore further studies should include multivariable analysis to take into account the possible effects of other parameters.

In the case of infection with both viruses, the absence of lesions was rarely observed. This study shows that, in FCV infection, the most common lesions are oral lesions (47%) and this observation correlates with previous studies.[2] On the other hand, in FeHV-1 infection, respiratory lesions are often observed (38%). Thirty-one per cent of sampled cats did not show any clinical sign in spite of positive PCR diagnosis for viral infection. In most rescue shelters, cats with clinical signs go into quarantine then arrive, whereas in the absence of clinical signs, they are directly put into contact with other cats. This situation allows a quick propagation of the infections from subclinical shedders.[24]

As the high prevalence of FCV and FeHV-1 infections associated with oral and respiratory signs are observed in rescue shelters, hygienic measures and prevention play a pivotal role to control diseases in such facilities,[25] where the cat population is very heterogeneous and where the vaccination and infection status of introduced animals are unknown.

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