



# Prevalence of feline herpesvirus-1, feline calicivirus, *Chlamydophila felis* and *Mycoplasma felis* DNA and associated risk factors in cats in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis

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Mireia Fernandez<sup>1</sup>, Edgar G Manzanilla<sup>1</sup>, Albert Lloret<sup>1</sup>,  
Marta León<sup>2</sup> and Jean-Christophe Thibault<sup>3</sup>

## Abstract

**Objectives** Our objective was to perform the first multicentric study in Spain to evaluate the prevalence of feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), *Chlamydophila felis* and *Mycoplasma felis* in cats with upper respiratory tract disease (URTD), conjunctivitis and/or gingivostomatitis (GS) compared with control cats; and to evaluate risk factors for these clinical conditions.

**Methods** Conjunctival and oropharyngeal swabs were collected and a questionnaire regarding signalment, lifestyle, vaccination history and clinical signs was obtained for each cat. Swabs were tested for each pathogen by real-time PCR.

**Results** The study population consisted of 358 cats, including 98 control cats. Among the 260 diseased cats, 127 cats presented with URTD, 149 cats had conjunctivitis, 154 cats were suffering GS; many cats presented more than one clinical condition. The prevalence observed of FHV-1, FCV, *C felis* and *M felis* was, respectively, 28.3%, 48.0%, 20.5% and 46.5% in cats with URTD; 24.2%, 43.6%, 19.5% and 38.3% in cats with conjunctivitis; and 15.6%, 58.4%, 9.1% and 37.7% in cats with GS. Prevalences in the control group were 6.1%, 15.3%, 2.0% and 20.4%, respectively. Coinfections were common among all groups of cats. Risk factors were identified for all groups. FHV-1, FCV and *C felis* were associated with URTD and conjunctivitis. FCV was strongly associated with GS. *M felis* was present in a high percentage of the population in all groups, but its role in these clinical conditions remains uncertain. Vaccination was protective for URTD and GS but not for conjunctivitis.

**Conclusions and relevance** This epidemiological study describes, for the first time, prevalence for FHV-1, FCV, *C felis* and *M felis* in Spain. In general, the prevalences found are similar to those reported in other countries. Factors associated with disease expression were also identified, which are relevant for practitioners.

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## Introduction

Upper respiratory tract disease (URTD) is a common condition, especially in cats living in communities or in close contact with other cats.<sup>1</sup> The disease is multifactorial and several pathogens are involved, the main ones being feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV), but other pathogens such as *Bordetella bronchiseptica*, *Chlamydophila felis* or *Mycoplasma* species are known or suspected to play a role in the disease.<sup>1</sup> Clinical signs

<sup>1</sup>Hospital Foundation Veterinary Clinic, Autonomous University of Barcelona, Bellaterra, Spain

<sup>2</sup>Meriel Laboratories, Barcelona, Spain

<sup>3</sup>Meriel SAS, Lyon, France

### Corresponding author:

Mireia Fernandez DVM, Hospital Foundation Veterinary Clinic, Autonomous University of Barcelona, Edifici V, Campus, 08191 Bellaterra, Spain  
Email: mfernandezara@gmail.com

in URTD can be similar, regardless of the pathogen involved.<sup>1</sup>

FHV-1 typically causes upper respiratory and ocular disease. Corneal dendritic ulcers are considered pathognomonic for this infection.<sup>2</sup> FCV produces acute oral and respiratory disease. The appearance of oral ulcers in a cat is suggestive of an infection with FCV. A limping syndrome associated with FCV is also described, with lameness and fever occurring shortly after the oral or respiratory infection.<sup>3</sup> *C felis* is a conjunctival pathogen and produces primarily severe conjunctivitis, but some cats can also manifest sneezing and nasal discharge.<sup>4</sup> *Mycoplasma felis* is part of the normal flora in the upper respiratory tract of the cat. Although its true role as a pathogen is not completely established, there is growing evidence of its association with conjunctivitis and URTD.<sup>5,6</sup>

Feline chronic gingivostomatitis (GS) is a severe chronic inflammation of the oral mucosa. Cats affected with this disease present with extreme pain, pseudoanorexia, sialorrhoea, loss of grooming and pawing at the mouth.<sup>7</sup> Despite being a frequent disease, the aetiopathogenesis is still unknown. It is believed to be a multifactorial disease, where some infections could play a role. Recently, several studies have correlated an increased prevalence of infection with FCV in cats with feline chronic GS.<sup>7–11</sup>

This study had two main objectives. The first was to evaluate, for the first time in Spain, the prevalence of FHV-1, FCV, *C felis* and *M felis* in a population of cats with signs of URTD, conjunctivitis or GS compared with a control population. The second objective was to study risk factors for the presence of clinical conditions and severity of disease. The main hypotheses were that the associations among pathogens and clinical syndromes reported in other countries would be similar in Spain; that *M felis* has a role in URTD and conjunctivitis; and, finally, that lack of a proper vaccination would be a risk factor for the presence and severity of disease.

## Materials and methods

### Study population

A cross-sectional study was conducted that included 30 veterinary practices in which practitioners were members of a national feline medicine study group (Grupo de Estudio de Medicina Felina de España [GEMFE AVEPA]) in Spain between September 2010 and January 2014. Each centre was asked to enrol between 10 and 20 cats. Inclusion criteria were the presence of clinical signs suggestive of URTD, conjunctivitis or GS. A control group was also recruited from some of the clinics recruiting cases in the different regions, which consisted of cats with none of the above-mentioned clinical conditions. A questionnaire for each cat included in the study was completed with information regarding signalment,

lifestyle, vaccination history, description and severity of clinical signs.

### Sample collection and testing

From each cat, two conjunctival and two oropharyngeal swabs were obtained and sent refrigerated in sterile dry containers to the laboratory for analysis. Real-time PCR was performed on pooled conjunctival and oropharyngeal samples from each cat for the detection of FHV-1, FCV, *C felis* and *M felis*.

Total nucleic acid was extracted from swab samples using the QIAamp DNA Blood BioRobot MDx Kit on an automated platform (BioRobot Universal; Qiagen), according to the manufacturer's instructions with slight modifications. Real-time PCR was performed at Vet Med Labor GmbH, a division of IDEXX Laboratories in Ludwigsburg (Germany), using the LightCycler 480 system (Roche) with proprietary forward and reverse primers and hydrolysis probes.

Target genes for pathogen detection using real-time PCR were as follows: FHV-1 *B* gene (S49775), FCV 5' *UTR* gene (DQ424892), *C felis ompA* gene (AP006861) and *M felis* 16S rRNA gene (AY741674). The molecular diagnostics were performed with six quality controls, including a PCR-positive control of known quantity, a PCR-negative control, a negative extraction control, an internal positive control spiked into the lysis solution to monitor the nucleic acid extraction efficiency and presence or absence of inhibitory substances, an RNA quality control and an environmental contamination monitoring control.

### Predictor and outcome variables

Outcome variables were clinical condition (all disease, URTD, conjunctivitis, GS) and severity of disease. The all disease group includes all those cats presenting any of the clinical conditions.

Predictor variables assessed included age (kitten: 0–0.5 years, junior 0.5–2 years, adults 2–10 years and senior >10 years); sex and neuter status; breed (purebred vs non-purebred); type of confinement (strictly indoor vs outdoor access); number of cats in the household (1, 2–6, >6); known proper vaccination; and pathogen status. Proper vaccination was considered if the adult cat had received core vaccinations (including FCV, FHV-1 and feline panleukopenia virus) in the previous 2 years and in the case of kittens and junior cats, if they had received two or three doses of primovaccination and first booster as indicated by age.

The diseased cats were further characterised according to severity of clinical signs. Rectal temperature, hyporexia and lethargy were recorded. Sneezing was graded 0–2 as none, occasional or frequent, respectively. Nasal and eye discharge were graded 0–4 as none, slight, medium, severe or very severe, respectively. Lymph

nodes size was classified 0–2 as all normal, only submandibular lymph nodes enlarged or other lymph nodes enlarged, respectively. Stomatitis was graded 0–4 as none, slight reddening, moderate-to-severe reddening, severe reddening with erosions or severe reddening with ulcers, respectively.

### Statistical analysis

All statistical analyses were performed using SAS 9.2. An individual cat was considered as the experimental unit, and the alpha level for determination of significance was 0.05 in all cases. Two sets of analyses were carried out using clinical condition or severity of clinical signs as outcome variables. The data set for each clinical condition only included animals with no clinical signs as the control group, and the data set for severity of clinical signs only included those animals presenting clinical signs and no control group.

Predictor variables were age, sex and neuter status, breed, lifestyle, number of cats in the household, proper vaccination and positive pathogen status. The relation between each outcome and predictor variable was calculated by univariate analysis using  $\chi^2$ /Fisher's exact test in the case of categorical variables and by ANOVA for continuous variables.

Multivariable analysis of the clinical condition was carried out using logistic regression. Multivariable analysis of severity of the clinical signs was carried out by multiple linear regression for rectal temperature, and by generalised linear models for all the scores. Variables were selected for inclusion in the regression analysis if the *P* value calculated for their association with the outcome variable in the univariable analysis was  $\leq 0.25$  and the variable was considered to be biologically significant. In case of co-linearity among predictors, the one to be included was selected based on its association with the outcome in the univariate analysis. The model was fitted by manual stepwise procedure. Veterinary centre of origin was initially considered in the models as a random factor but was removed as it had no significant effect ( $P > 0.25$ ).

## Results

### Characteristics of the study population

The study population consisted of 358 cats. The control group included 98 cats. The disease group included 260 cats, with 127 (35.5%) presenting with URTD, 149 (41.6%) with conjunctivitis and 154 (43.0%) with GS.

The whole population was composed of 74 (21.1%) entire males, 105 (29.9%) neutered males, 91 (25.9%) entire females and 81 (23.1%) neutered females. Most cats were not purebred (76.9%). Mean age was 3.73 years, with 96 (28.1%) kittens, 99 (28.9%) juniors, 121 (35.4%) adults and 26 (7.6%) seniors. Fifty-six percent of the cats were strictly indoor cats, whereas 44% had some

outdoor access. Considering the number of cats within the household, 107 (31.9%) of them lived alone, 143 (42.7%) were in a household with 2–6 cats and 85 (25.4%) lived with more than six cats. From the whole population, only 46.1% of the cats were considered properly vaccinated.

On physical examination of the diseased cats, mean rectal temperature was 38.3°C, only 67/260 (25.8%) had lethargy and 65/260 (25.0%) presented with hyporexia. Stomatitis grading was 0 in 38.1%, 1 in 14.3%, 2 in 19.8%, 3 in 14.7% and 4 in 13.1% of the diseased cats, with a mean of 1.50. Grade of sneezing was 0 in 42.3%, 1 in 30.0% and 2 in 27.7% of the diseased cats, with a mean of 0.85. Nasal discharge grading was 0 in 51.8%, 1 in 16.5%, 2 in 19.4%, 3 in 8.3% and 4 in 4.0% of the diseased cats, with a mean of 0.96. Eye discharge grading was 0 in 37.9%, 1 in 24.9%, 2 in 18.2%, 3 in 14.7% and 4 in 4.3% of the diseased cats, with a mean of 1.23. Conjunctivitis grading was 0 in 44.7%, 1 in 16.2%, 2 in 20.6%, 3 in 13.8% and 4 in 4.7% of the diseased cats, with a mean of 1.18. Grading of lymph node abnormalities was 0 in 57.3%, 1 in 40.3% and 2 in 2.4% of the diseased cats, with a mean of 0.45.

### Prevalence and correlation of infection and clinical conditions

Overall, FHV-1 was detected in 54 (15.1%) samples, FCV in 144 (40.2%), *C felis* in 37 (10.3%) and *M felis* in 117 (32.7%). The prevalence of infection for each clinical condition is shown in Table 1. The prevalence of all pathogens was significantly higher in each of the disease groups than in the control group in the univariate analysis.

Negative results for all pathogens were obtained in 143 (39.9%) samples. From these cats, 45% were controls, 23% had URTD, 34% had conjunctivitis and 30% had GS (Figure 1). Positive FHV-1 status was significantly related to URTD (67%;  $P < 0.001$ ) and conjunctivitis (67%;  $P < 0.001$ ) but not to GS (44%;  $P = 0.882$ ). Positive FCV status was significantly related to GS (63%;  $P < 0.001$ ) but was not related to URTD (45%;  $P = 0.062$ ) or conjunctivitis (42%;  $P = 0.325$ ). Positive *C felis* status was significantly related to URTD (70%;  $P < 0.001$ ) and conjunctivitis (78%;  $P < 0.001$ ) but not to GS (38%;  $P = 0.600$ ). And, finally, positive *M felis* status was significantly related to URTD (50%;  $P < 0.001$ ) but did not attain significance for conjunctivitis (49%;  $P = 0.068$ ) or GS (50%;  $P = 0.087$ ).

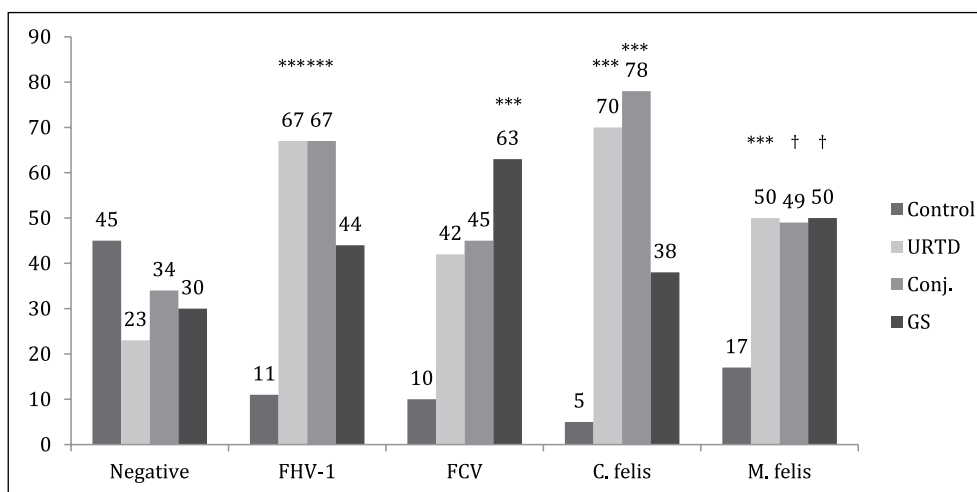
Coinfections were very common, occurring in 28.2% of all samples tested and 47.0% of all positive samples. In the control group, coinfections were present in 9% of the cats and 23.5% of the positive samples. In the diseased cats, coinfections were present in 35.4% of the total and 50.8% of the positive samples. Coinfection was more frequent in animals showing all three clinical conditions (71% of co-infected) than in those showing two (35%) or one (28%) of the clinical conditions.

**Table 1** Prevalence and univariate analysis of each pathogen for each one of the clinical conditions

	Control (n = 98)	All disease (n = 260)	URTD (n = 127)	Conjunctivitis (n = 149)	GS (n = 154)
<b>FHV-1</b>					
Positive	6 (6.1)	48 (18.5)	36 (28.3)	36 (24.2)	24 (15.6)
P value		<b>0.004</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.024</b>
<b>FCV</b>					
Positive	15 (15.3)	129 (49.6)	61 (48.0)	65 (43.6)	90 (58.4)
P value		<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
<b>Chlamydomphila felis</b>					
Positive	2 (2.0)	35 (13.5)	26 (20.5)	29 (19.5)	14 (9.1)
P value		<b>0.002</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.025</b>
<b>Mycoplasma felis</b>					
Positive	20 (20.4)	97 (37.3)	59 (46.5)	57 (38.3)	58 (37.7)
P value		<b>0.002</b>	<b>&lt;0.001</b>	<b>0.003</b>	<b>0.003</b>

Data are n (%). Bold indicates significant P value

URTD = upper respiratory tract disease; GS = gingivostomatitis; FHV-1 = feline herpesvirus-1; FCV = feline calicivirus



**Figure 1** Percentages of cats presenting each clinical condition according to the positivity status for each one of the studied pathogens. Bars marked with symbols for each one of the pathogens are significantly different from the corresponding bar in the negative group (\*\*\*)  $P < 0.001$ ; †  $P < 0.1$ ). UR TD = upper respiratory tract disease; Conj = conjunctivitis; GS = gingivostomatitis

#### Univariate and multivariable assessment of risk factors for the clinical conditions

Table 2 shows the univariate analysis of the risk factors for each of the disease groups. Age, sex, neutering status, being purebred, being kept indoors, number of cats in the household and vaccination history were variably associated with all groups. In the multivariable analysis (Table 3), only the following factors remained significant: UR TD was significantly associated with positive results for FHV-1, FCV and *C felis* (in addition to being male, purebred and living in a multi-cat household); conjunctivitis was significantly associated with positive results for FHV-1, FCV and *C felis* (in addition to being purebred, having access to the outdoors and living in a multi-cat household); and GS was significantly

associated with positive results for FCV and *C felis* (in addition to being older, male, purebred and living in a multi-cat household). Not being properly vaccinated was a significant risk factor for UR TD and GS.

#### Multivariable assessment of risk factors for the severity of disease

Significant predictors for severity of each clinical sign are summarised in Table 4. FHV-1 was significantly related to lethargy, and more severe sneezing, nasal and ocular discharge, and conjunctivitis. FCV was significantly related to stomatitis, hyporexia, lethargy and more severe enlarged lymph nodes. *Chlamydomphila felis* was only significantly related to more severe ocular discharge and conjunctivitis. Finally, *M felis* was

**Table 2** Univariate analysis of the studied risk factors for each one of the clinical conditions

	Control (n = 98)	All disease (n = 260)	URTD (n = 127)	Conjunctivitis (n = 149)	GS (n = 154)
Age					
Kitten	25/95 (26.3)	71/255 (27.8)	55/126 (43.7)	60/148 (40.5)	18/150 (12.0)
Junior	38/95 (40.0)	61/255 (23.9)	28/126 (22.2)	38/148 (25.7)	39/150 (26.0)
Adult	24/95 (25.3)	97/255 (38.0)	37/126 (29.4)	45/148 (30.4)	70/150 (46.7)
Senior	8/95 (8.4)	26/255 (10.2)	6/126 (4.8)	5/148 (3.4)	23/150 (15.3)
<i>P</i> value		<b>0.024</b>	<b>0.008</b>	<b>0.015</b>	<b>&lt;0.001</b>
Sex					
Male	42/98 (42.9)	144/260 (55.4)	76/127 (59.8)	76/149 (51.0)	90/154 (58.4)
Female	56/98 (57.1)	116/260 (44.6)	51/127 (40.2)	73/149 (49.0)	64/154 (41.6)
<i>P</i> value		<b>0.033</b>	<b>0.011</b>	0.210	<b>0.016</b>
Neuter status					
Neutered	56/98 (57.1)	130/253 (51.4)	46/124 (37.1)	52/146 (35.6)	95/148 (64.2)
Entire	42/98 (42.9)	123/253 (48.6)	78/124 (62.9)	94/146 (64.4)	53/148 (35.8)
<i>P</i> value		0.288	<b>0.002</b>	<b>&lt;0.001</b>	0.266
Purebred					
Yes	12/98 (12.2)	71/258 (27.5)	30/125 (24.0)	41/147 (27.9)	50/154 (32.5)
No	86/98 (87.8)	187/258 (72.5)	95/125 (76.0)	106/147 (72.1)	104/154 (67.5)
<i>P</i> value		<b>0.005</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.005</b>
Indoor (strict)					
Yes	68/98 (69.4)	129/256 (50.4)	57/125 (45.6)	69/148 (46.6)	79/150 (52.7)
No	28/98 (28.6)	127/256 (49.6)	68/125 (54.4)	79/148 (53.4)	71/150 (47.3)
<i>P</i> value		<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.005</b>
Number of cats					
1	52/89 (58.4)	55/246 (22.4)	25/120 (20.8)	34/138 (24.6)	36/149 (24.1)
2–6	21/89 (23.6)	122/246 (49.6)	57/120 (47.5)	65/138 (47.1)	72/149 (48.3)
>6	16/89 (18.0)	69/246 (28.0)	38/120 (31.7)	39/138 (28.3)	41/149 (27.5)
<i>P</i> value		<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Proper vaccination					
Yes	57/98 (58.2)	108/260 (41.5)	46/127 (36.2)	72/149 (48.3)	59/154 (38.3)
No	41/98 (41.8)	152/260 (58.5)	81/127 (63.8)	77/149 (51.7)	95/154 (61.7)
<i>P</i> value		<b>0.009</b>	<b>0.001</b>	0.130	<b>0.002</b>

Data are n (%). Bold indicates significant *P* value

URTD = upper respiratory tract disease; GS = gingivostomatitis

**Table 3** Odds ratio and 95% confidence intervals for the risk factors found to be significant in the multivariable analysis for each clinical condition

Predictor variables	All disease	URTD	Conjunctivitis	GS
Age, adult vs kitten				8.2 (2.6–26.1)**
Male	2.2 (1.2–4.1)*	3.0 (1.4–6.4)**		3.3 (1.5–7.2)**
Purebred	4.0 (1.8–8.9)***	4.9 (1.8–13.4)**	3.8 (1.7–8.6)**	5.1 (2.0–13.0)***
Outdoor			2.6 (1.1–6.1)*	
Number of cats, 2–6 vs 1	5.7 (2.8–11.5)***	7.2 (2.8–18.1)***	3.8 (1.8–8.0)**	6.5 (2.6–16.2)***
Lack of proper vaccination	2.7 (1.4–5.1)**	4.0 (1.7–9.6)***		2.7 (1.1–6.4)*
FHV-1-positive		3.2 (1.1–9.6)*	3.3 (1.2–9.3)*	
FCV-positive	5.3 (2.6–11.0)***	4.9 (2.0–11.8)***	3.1 (1.4–7.2)**	13.9 (5.3–36.1)***
<i>C felis</i> -positive	20.8 (2.4–182)**	59.3 (5.5–635)***	27.2 (3.3–222)**	12.3 (1.1–134)*

Data are odds ratio (95% confidence interval)

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001

URTD = upper respiratory tract disease; GS = gingivostomatitis; FHV-1 = feline herpesvirus-1; FCV = feline calicivirus

**Table 4** Odds ratio and 95% confidence intervals for the risk factors found to be significant in the multivariable analysis for severity of disease

Clinical sign	Significant predictors	Values		
Lethargy	FHV-1**	2.7 (1.3–5.5)		
	FCV*	2.0 (1.0–3.8)		
Hyporexia	Purebred*	0.45 (0.20–1.02)		
	Lack of proper vaccination*	2.22 (1.02–5.00)		
	FCV**	2.4 (1.2–4.7)		
Stomatitis (0–4 score)	Age*** (vs kitten)	Junior	Adult	Senior
		8.0 (3.6–17.9)	12.1 (5.7–25.7)	25.4 (9.3–69.5)
Sneeze (0–2 score)	FCV***	4.1 (2.4–7.1)		
	Age*** (vs kitten)	Junior	Adult	Senior
		0.38 (0.19–0.78)	0.20 (0.10–0.38)	0.08 (0.03–0.23)
Nasal discharge (0–4 score)	Indoor*	1.96 (1.11–3.44)		
	FHV-1**	2.18 (1.24–3.85)		
	<i>M felis</i> *	2.87 (1.45–5.65)		
	Age*** (vs kitten)	Junior	Adult	Senior
		0.35 (0.17–0.71)	0.19 (0.10–0.37)	0.07 (0.02–0.23)
Ocular discharge (0–4 score)	Proper vaccination**	1.85 (1.06–3.23)		
	FHV-1**	2.73 (1.38–5.41)		
	<i>M felis</i> ***	2.53 (1.46–4.59)		
	Age*** (vs kitten)	Junior	Adult	Senior
		0.24 (0.12–0.50)	0.23 (0.12–0.43)	0.07 (0.02–0.22)
Conjunctivitis (0–4 score)	FHV-1***	3.88 (1.98–7.52)		
	FCV***	0.38 (0.22–0.63)		
	<i>C felis</i> *	2.07 (1.01–4.26)		
	Age*** (vs kitten)	Junior	Adult	Senior
		0.29 (0.14–0.58)	0.19 (0.10–0.38)	0.07 (0.02–0.25)
Lymph nodes (0–2 score)	FHV-1***	3.80 (1.96–7.25)		
	<i>C felis</i> ***	3.38 (1.96–7.35)		
	FCV**	2.22 (1.28–3.85)		

Data are odds ratio (95% confidence interval)

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

FHV-1 = feline herpesvirus-1; FCV = feline calicivirus

significantly related to more severe sneezing and nasal discharge. Age was inversely related to severity of most clinical signs, while it was related to more severe GS. Finally, lack of proper vaccination was related to more hyporexia and more severe nasal discharge.

## Discussion

This is the first epidemiological study of feline upper respiratory pathogens in Spain. The reported prevalence in other countries has been variable and results depend, among other factors, on the specific technique used and the population of cats studied. The present study aimed at reporting prevalence in a wide segment of the population and therefore it was designed as a multicentric study that included cats from single and multi-cat households, privately owned and free-roaming cats included in a trap–neuter–return (TNR) programme, from several regions in Spain.

Prevalence data obtained in the control group is in agreement with those previously reported. FHV-1 was detected in 6% of control cats. Previous studies based on PCR have reported a wide range of results, from 2.6% in privately owned cats in the USA to 63% in a shelter in Korea.<sup>6,12</sup> Consideration must be given to the fact that FHV-1 is shed intermittently and remains latent in the trigeminal ganglia most of the time, undetectable by PCR.<sup>13</sup> FCV was detected in 15% of control cats. Prevalences of 0% to 29% have been reported in healthy asymptomatic cats.<sup>11,12,14–16</sup> *Chlamydophila felis* was only detected in 2% of control cats. Most studies have previously reported null prevalence in healthy cats,<sup>6,12,17,18</sup> while two multicentric studies performed in shelters reported higher prevalences of 3.0% and 15.4%, respectively.<sup>15,17</sup> Finally, *M felis* is the least studied of the four pathogens. As it is a known commensal of the feline upper respiratory tract, prevalence in healthy cats is higher than the other pathogens.<sup>1</sup> Our study showed a

prevalence of 20%, while another study performed in shelters in the USA obtained prevalences of 0–19.4%, depending on the shelter examined.<sup>17</sup>

In total, 127 cats with URTD were included in the study. Prevalence of the four pathogens was higher in the URTD group than in the control cats, and these differences were significant in the multivariable analysis, with the exception of *M felis*. Whether these pathogens were primarily responsible for the clinical signs, concomitant infections or appeared secondarily to the disease could not be established. As expected, FHV-1 infection was associated with increased severity of respiratory and ocular signs. Interestingly, *M felis* was significantly associated with increased severity in nasal discharge and sneezing, suggesting some role in these respiratory signs, either as a primary pathogen or, more likely, as an opportunistic secondary infection. Males had a three times higher risk of URTD than females, as reported previously in URTD and FHV-1 infection.<sup>13,19,20</sup> The reason for this sex predisposition is unknown. In other studies, intact males were predisposed and it was assumed that they would be more likely to go outdoors and more likely to be exposed to contagious agents. However, based on our results, strictly indoor cats do not seem to be protected from infection and, therefore, the results do not support this hypothesis. However, living in a multi-cat household was, in fact, an important risk factor, as previously described, with cats from a multi-cat household having 7.2 times more of a risk of developing URTD.<sup>15</sup> Purebred predisposition was also demonstrated in cats entering a UK shelter, although a reason for this finding is not clear.<sup>21</sup>

In total, 149 cats with conjunctivitis were included in the study. Prevalence of the four pathogens was also higher than in control cats, and these differences were again significant in the multivariable analysis for conjunctivitis, except for *M felis*. Many of the cats with conjunctivitis also had signs of URTD and therefore there is great overlap between the two groups, which could explain the apparent relationship between FCV and conjunctivitis. As expected, *C felis* was associated with severity of ocular signs. The role of *M felis* as a primary pathogen in conjunctivitis is still debated.<sup>6,17,22–26</sup> Results from our study do not support this association. Although cats in the conjunctivitis group had *M felis* more commonly detected than cats in the control group (38.3% vs 20.4%), this difference did not remain significant in the multivariable analysis and *M felis* was not associated with increased severity of ocular signs. Being purebred was a significant risk factor for conjunctivitis. However, no breed predilection has been found in previous studies, and, again, the reason for this finding is not clear.<sup>4,27</sup> Surprisingly, young age was not significant in the multivariable analysis. Conjunctivitis is commonly found in young cats, and most of the time it is associated with

*C felis* or FHV-1.<sup>4,27,28</sup> Contact with other cats seems of special importance in conjunctivitis, as being allowed outdoors and living with other cats were significant risk factors.

Finally, 154 cats with GS were included in the study. In this population, the prevalence of FCV was much higher than in control cats and the highest of all groups. FCV was significantly associated with GS and was related to general signs of malaise such as hyporexia, increased lymph nodes or lethargy, as well as being associated with severity of stomatitis. The increased severity of hyporexia is probably related to the presence of lesions in the oral cavity, frequently associated with this viral infection.<sup>3</sup> Although some previous studies did not find a correlation between FCV and chronic feline GS, there is growing evidence of a link between the two.<sup>7–11</sup> However, only 58% of the cats with GS were positive for FCV and, therefore, there is still a large proportion of cats with GS that cannot be explained by FCV infection alone. Other infectious diseases, as well as the cat's own immune response, have been implicated.<sup>11,29</sup> *C felis* was also, surprisingly, significantly associated with GS, although the confidence interval for the odds ratio has a lower limit close to 1, which could indicate a weaker association. Male, purebred and older cats were associated with GS. Feline chronic GS is known to be a disease of mature cats, but no sex and breed predispositions have been reported.<sup>29</sup> Cats not living alone were 6.5 times more likely to develop GS. The reason for this finding is not obvious but could relate to the increase risk of FCV infection and the development of escaping quasi-species in multi-cat environments.<sup>30,31</sup>

The lack of a proper vaccination protocol was a risk factor for cats with URTD and GS. However, it was not associated with conjunctivitis. This could be explained because of the important relationship between conjunctivitis and *C felis*, with cats with *C felis* being much more likely to present conjunctivitis. Vaccination against *C felis* is considered non-core and, therefore, it was not included in the definition of proper vaccination.<sup>32</sup> Twenty-three percent of the cats properly vaccinated were not vaccinated against *C felis*, and this might explain the apparent lack of efficacy of vaccination in preventing conjunctivitis. Looking at severity of disease, vaccination protected against hyporexia and reduced the severity of nasal discharge. However, it did not protect against ocular discharge and conjunctivitis, probably for the same reasons mentioned above. It is worth mentioning that the definition of properly vaccinated adult cats included only those that had received a booster in the past 2 years. Current guidelines for vaccination of cats recommend tri-annual revaccination for the general feline population, but some guidelines recommend more frequent boosters (ie, annual) in cats at high risk. Therefore, 2 years were chosen arbitrarily to improve the ability to

detect an effect of vaccination in the whole population.<sup>2,3,32,33</sup>

The study has some limitations. First, PCR was performed on pooled samples from conjunctival and oropharyngeal mucosa. It would have been interesting to obtain separate results but it would have increased costs of the study. Second, although questionnaires were obtained with all the samples, some of the information was missing, especially in animals from TNR programmes where no vaccination history or age was known. Finally, the questionnaire was not designed to differentiate cats with acute stomatitis usually associated with FCV infection from cats with chronic feline GS syndrome, and they were all grouped together in the GS group. However, most of the cats in this group were suffering from the chronic form and therefore conclusions from this group may be extrapolated to cats suffering chronic feline GS syndrome.

## Conclusions

This epidemiological study describes, for the first time, prevalence for FHV-1, FCV, *C felis* and *M felis* infections in Spain. These prevalences are, in general, similar to prevalences reported in other countries. Some risk factors are described for these clinical conditions. FHV-1, FCV and *C felis* were associated with URTD and conjunctivitis. FCV was strongly associated with GS. *M felis* was present in a high percentage of the population in all groups, but its role in these clinical conditions remains uncertain. Finally, proper core vaccination (as defined above) was protective for URTD and GS but not for conjunctivitis.

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