Article

Descriptive epidemiology of upper respiratory disease and associated risk factors in cats in an animal shelter in coastal western Canada

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Abstract – We examined 250 cats at an animal shelter in the coastal temperate region of Canada to determine whether age, source, gender, and sterilization status influenced risk of shedding at intake, transmission of infection, and development of clinical upper respiratory disease (URD). On admission, 28% of the cats were positive for 1 or more infectious agent related to URD; 21% were carriers of *Mycoplasma felis* and < 3% were carriers of feline calicivirus (FCV), feline herpesvirus-1 (FHV-1) or *Bordetella bronchiseptica. Chlamydophila felis* and H1N1 influenza virus were not detected. Carrier status was not affected by source, gender, sterilization status, or age (P > 0.05). Viral and bacterial shedding increased by 9% and 11%, respectively, over 3 sampling times (days 1, 4, and 10). Over 40 days after admission, the cumulative probability of developing URD was 2.2 times greater for stray than owner-surrendered cats (P = 0.02) and 0.5 times as great for neutered cats as for intact cats (P = 0.03). Cats that were shedding at intake were 2.6 times more likely to develop URD than were non-carriers (P < 0.002). Cats with FHV-1 and *B. bronchiseptica* infections were most at risk compared with non-shedding cats (P < 0.01).

Résumé – Épidémiologie descriptive de la maladie respiratoire supérieure et facteurs de risque chez le chat dans un refuge situé dans la côte ouest du Canada. Nous avons examiné 250 chats dans un refuge de la région côtière tempérée du Canada. Nous avons déterminé la présence d'infection latente chez les chats de provenance diverses, par âge, par sexe (castré ou non-castré) lors de leur arrivé au refuge. Nous avons aussi étudié la transmission des pathogènes et le développement de symptômes rhinosinusites pendant leur séjour (40 jours). Au prélèvement du premier écouvillonnage, 21 % était positif pour le Mycoplasme felis (*M. Felis*) et moins de 3 % était positif pour le calicivirus félin (FCV), l'herpèsvirus félin de type 1 (FHV1) ou le Bordetella *bronchiseptica.* Ni Chlamydophila felis (*C. felis*) ni H1N1 n'ont été dépisté. Le nombre de porteurs latents n'était pas affecté par l'origine des chats, le sexe ou l'âge (P > 0,05). La probabilité cumulée de développer des symptômes de maladie était 2,64 fois supérieure pour les chats de maison (P = 0,02) et 0,5 fois supérieure pour les chats castrés que pour les chats non castré (P = 0,03). En particulier, les porteurs de FHV1 et *B. bronchiseptica* étaient plus à risque que les chats non-porteurs (P < 0,01). Nous avons conclu que les chats avec une infection latente de FHV1 ou *B. Bronchiseptica*, les chats errants et les chats castrés étaient plus vulnérables a la maladie des voies respiratoires supérieures dans ce refuge.

(Traduit par les auteurs)

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Introduction

U pper respiratory disease (URD) is the primary health issue reported in cats during their stay in animal shelters (1) and post adoption (2,3). In shelters, URD is an important cause of morbidity due in part to poor ventilation, stress-induced

immunosuppression, and overcrowding which complicate management of disease (4). Upper respiratory tract disease (URD) is the primary health reason for euthanasia of kittens in animal shelters (5) and cats receiving treatment are subjected to extended periods of confinement with minimal human

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interaction (6). Outbreaks of URD are common in animal shelters (7), which together with the day-to-day management of sick cats represent a significant financial burden for humane organizations.

The feline respiratory disease complex involves a variety of pathogens. Feline herpesvirus-1 (FHV-1) and feline calicivirus (FCV) are believed to be responsible for most cases of URD in animal shelters and are followed by Mycoplasma felis, Bordetella bronchiseptica, and Chlamydophila felis (8). In March 2009, a new human influenza A H1N1 virus emerged in Mexico and the United States (9). From fall 2009 to early 2010 in the United States there were several reports of H1N1 influenza virus in animals, 2 of which were owned cats believed to have contracted the virus from their owners (10). Feline herpesvirus-1, which has a prevalence of between 0.2% and 33% in household cats (11) has been reported at rates between 63% and 84% in shelter cats in South Korea (12), Belgium (13), and California, USA (14). Feline calicivirus, which is present in about 8% of household cats (14), is believed to affect about 40% of shelter cats (15). Stray cats admitted to shelters are a known source of these pathogens (16) and there are equivocal results as to whether age, gender, and sterilization status are potential risk factors (14,17,18). However, scientists agree that identifying the characteristics of cats at greater risk for developing URD is critical to the management of the disease in animal shelters (7,19,20).

Despite a substantial body of knowledge on prevalence of URD and associated risk factors in animal shelters worldwide, the epidemiology of URD in Canada, particularly in the coastal temperate climatic region which exists in British Columbia, has not been examined. The Canadian Federation of Humane Societies (21) estimates that there are about 150 Humane Societies/Societies for the Prevention of Cruelty to Animals across Canada, each managing many shelters, in addition to many private rescue organizations and animal control agencies. The present study examined the prevalence of subclinical upper respiratory infections in cats upon admission to an animal shelter, and the risk factors associated with the subsequent spread of infection and development of URD over time.

Materials and methods

The study took place at the Vancouver Branch of the British Columbia Society for the Prevention of Cruelty to Animals (BC SPCA), Canada, between May and November 2010. It was part of a research project to examine emotional and immunological changes in anxious, frustrated, or content cats when provided with behavioral interventions. During the first 10 d of the study, cats were housed in a pre-adoption housing unit with limited access to the public.

Cats were housed individually in stainless steel cages (76 \times 76 \times 71 cm) furnished with litter boxes, stainless steel food and water bowls, and bedding. Age-appropriate food (Hill's Pet Nutrition, Mississauga, Ontario) and water was provided twice per day (07:00 h and 17:00 h). Windows provided natural light and temperature was maintained at 20°C \pm 2°C. The shelter had 6 separate housing areas with a maximum capacity to house 120 cats. The facility also included an isolation area for sick cats and in-house medical staff at the on-site veterinary hospital.

	Total (N = 250)	FCV	%	FHV	%	M. felis	%	B. b	%
Female	138	5	71	2	40	24	44	3	50
Male	112	2	29	3	60	30	56	3	50
OS	125	2	29	0	0	30	56	3	50
Stray	125	5	71	5	100	24	44	3	50
Fixed	148	1	14	1	20	18	33	2	33
Intact	102	6	86	4	80	36	67	4	67
Juvenile	50	4	57	5	100	27	50	2	33
Adult	134	1	14	0	0	9	17	1	17
Senior	66	2	29	0	0	18	33	3	50

FCV — Feline calicivirus, FHV — Feline herpes virus-1, *M. felis — Mycoplasma felis, B. b — Bordetella bronchiseptica,* OS — owner-surrendered.

Table 2. Prevalence of shedding of various viruses and bacteria from ocular and pharyngeal swabs of shelter cats (N = 250) determined at intake (before vaccination, day 1), at day 4 and at day 10

	Day (N =	1 250)	Day (N =	4 233)	Day (N =	10 221)
	Ν	%	N	%	N	%
FCV	7	2.8	6	2.6	8	3.6
FHV-1	5	2	12	5.2	23	10.4
M. felis	52	20.8	49	21.0	69	31.2
C. felis	1	0.4	1	0.4	3	1.4
B. bronchiseptica	6	2.4	6	2.6	10	4.5
Viral-All	12	4.8	18	7.7	31	14.0
Bacterial-All	56	22.4	57	24.5	74	33.5

All samples were negative for H1N1 virus, FCV — Feline calicivirus, FHV-1 — Feline herpes virus-1, *M. felis — Mycoplasma felis, C. felis — Chlamydophila felis, B. bronchiseptica — Bordetella bronchiseptica.*

The shelter followed strict biosecurity measures, including spot cleaning of cages daily (removing debris and wiping cages with a clean cloth dipped in 1% Virkon[®] solution) and disinfection of cages between cats with a 2% Virkon[®] solution. Animal care staff did not wear gloves or protective gowns during cleaning of cages but washed their hands with a foaming alcohol handrub (MicrosanTM Encore; Deb Product, Waterford, Ontario) following contact with an animal. Cats with observed clinical signs of URD, such as sneezing, were immediately transferred to an isolation ward and received medical care.

Two-hundred and fifty cats that were either surrendered by their owners or brought in as strays by a humane officer were enrolled in the study. Age was provided by the owners or estimated by shelter staff, and categorized as juvenile (6 to 12 mo), adult (1 to 7 y), or senior (> 8 y). Swabs were obtained at intake and subsequently on days 4 and 10. The procedure was carried out by 1 of 3 Registered Animal Health Technicians using polymerase chain reaction (PCR) swabs according to IDEXX procedures. A sterile swab was rolled on the medial conjunctiva and another on the posterior oral-nasal pharynx. The swabs were placed into individual sterile transport tubes (ST RPLEX, Etobicoke, Ontario), refrigerated, and submitted for real-time polymerase chain reaction (RT-PCR) testing within 8 h. After the first swab, cats were vaccinated with a modified live vaccine (Fel-O-Guard+3 Boehringer Ingelheim, Burlington, Ontario) and dewormed (Strongid® T; Pfizer, Pointe-Claire, Quebec). Cats with observed clinical signs of URD, gingivitis, or injury

Table 3.	Multivariate	associations	of upper	respiratory	disease ii	n shelter	cats
(N = 250)) with charad	cteristics and	shedding	g status			

	Total	Cats without URD (%)	Cats with URD (%)	Odds ratio	95% Confidence limits for the odds ratio	<i>P</i> -value
Gender						
Female	138	96 (70)	42 (30)	1.00	0.50.0.0/	0 (0
Male	112	/0 (63)	42 (38)	1.28	0./0-2.34	0.43
Source						
OS	125	88 (70)	37 (30)	1.00		
Stray	125	78 (62)	47 (38)	2.21	1.14-4.29	0.02
Sterilization						
Intact	102	75 (74)	27 (26)	1.00		
Neutered	148	91 (61)	57 (39)	0.46	0.23-0.94	0.03
Age						
Adult	134	94 (70)	40 (30)	1.00		
Juvenile	50	34 (68)	16 (32)	1.49	0.67-3.31	0.33
Senior	66	38 (58)	28 (42)	1.66	0.78-3.55	0.19
Carrier state						
Non-carrier	162	125 (77)	37 (23)	1.00		
Carrier	88	41 (47)	47 (53)	2.64	1.42-4.90	< 0.002
FCV						
Negative	181	130 (72)	51 (28)			
Positive	7	4 (57)	3 (43)	1.90	0.027-11.69	0.46
FHV-1						
Negative	181	130 (72)	51 (28)			
Positive	5	0 (0)	5 (100)	Inf	2.23–Inf	0.002
R hronchiseptica						
Negative	181	130 (72)	51 (28)			
Positive	6	1 (17)	5 (83)	12.56	1.36-605.27	0.01
C felis						
Negative	181	130(72)	51 (28)			
Positive	1	0 (0)	1 (100)	Inf	0.06–Inf	0.27
M Eslis						
Negative	181	130(72)	51 (28)			
Positive	54	33(61)	21(39)	1.62	0.81-3.20	0.29
1 0011110	<i>J</i> 1	55 (01)	21 (37)	1.02	0.01-0.20	0.2)

Data were analyzed by analysis of covariance, adjusting for the following covariates: gender, source, sterilization status, and age. FCV — Feline calicivirus, FHV-1 — Feline herpes virus-1, *B. bronchiseptica* — *Bordetella bronchiseptica, C. felis — Chlamydophila felis, M. feli — Mycoplasma felis.* Inf — infinity.

at admission were not included in the study. Samples were analyzed for FHV-1, FCV, C. felis, M. felis, B. bronchiseptica, and influenza virus H1N1 by RT-PCR assays, that were based on IDEXX's oligonucleotides and protocols (22). Each test used a fluorescent probe that matched a unique segment of the organism's DNA or cDNA to ensure high specificity and sensitivity. Real-time PCR was performed with standard primer and probe concentrations using the Roche LightCycler® 480 Probes Master mastermix (Roche Applied Science, Indianapolis, Indiana, USA) and default cycling conditions on a Roche LC480 instrument and 384-well plate configuration.

Statistical analysis

Prevalence of subclinical infections was calculated as the number of cats without clinical signs of URD that were PCR positive for FHV-1, FCV, C. felis, M. felis, or B. bronchiseptica upon admission, divided by all cats in the study (N = 250). Multivariate logistic regression was used to examine the effects on the incidence of URD of gender (male versus female), source

[owner-surrendered (OS) versus stray], sterilization status (intact versus neutered), age (juveniles, adults, and seniors), and carrier state (shedding versus not shedding at intake for each specific pathogen). To determine the influence of the individual pathogen (FCV, FHV-1, B. bronchiseptica, C. felis, or M. felis) on the prevalence of URD, an analysis of covariance (ANCOVA) was performed in which the covariates were gender, source, sterilization, and age. These analyses were run on a subset of the 250 cats which included carriers of the particular pathogen and non-carriers of all pathogens (carriers of other pathogens were excluded from the analyses). Cats could be carriers for more than 1 pathogen, so some cats were included in more than 1 analysis. The P-values derived from a model adjusted for all other variables in the model are reported for these analyses.

A Kaplan-Meier survival analysis (23) compared the percentage risk of developing URD by days 7, 14, 21, and > 30(maximum of 40 d) according to characteristics such as carrier status, age, source, gender, and sterilization status. A Cox regression analysis using the survival package in R statistical

	Table 4. Cox Proportional Hazards Models and significance of the survival analysis
t	for each of the demographic characteristics

	N	Day 7 %	Day 14 %	Day 21 %	> 30 days %	Hazard ratio (CI)	$\Pr\left(> z \right)$
Gender							
Female	138	9.5	23	38	45		
Male	112	13	31	48	63	1.17 (0.72–1.93)	0.52
Source							
OS	125	7.3	19	33	45		
Stray	125	16.3	36	50	61	2.46 (1.48-4.09)	0.001
Gender status							
Neutered	148	15	30	45	53		
Intact	102	7	15	35	44	0.43 (0.23–0.78)	0.01
Age							
Adult	50	14	22	40	44		
Juvenile	134	19	27	44	63	1.85 (0.94-3.64)	0.07
Senior	66	11	30	45	60	1.19 (0.67–2.10)	0.56
Carrier state							
Non-carrier	162	2.7	13	28	41		
Carrier	88	29	46	55	76	2.39 (1.44-3.99)	< 0.001

N — total number; CI — confidence interval.

software was used to determine if the risk of contracting URD over time was significantly affected by these characteristics. All analyses were performed with R version 2.10.1. (24).

Results

Shedding rate at intake and over time

At intake, 28% (n = 69) of cats were carriers for 1 or more pathogens. Of the positive samples 22%, 2.8%, 2.0%, and 2.4% were positive for *M. felis*, FCV, FHV-1, and *B. bronchiseptica*, respectively. Co-infections with *M. felis* were identified for *B. bronchiseptica* and FCV for 3 and 1 of the samples, respectively. Of the samples obtained at admission, all were negative for *C. felis* and influenza H1N1 virus. Risk of being a carrier was not significantly affected by gender, source, sterilization status, or age (Fisher's exact text P > 0.05) (Table 1). Subsequent swabs (days 4 and 10) showed an increase of 9% and 11% over the 10 d in viral and bacterial infections, respectively. Feline calicivirus (1%), *B. bronchiseptica* (3%), and *C. felis* (1%) showed lower increases than FHV-1 (8%) and *M. felis* (10%) (Table 2). All cats remained negative for influenza H1N1 virus throughout the study.

Risk factors associated with the development of URD

As shown in Table 3, gender was not a significant factor for the development of URD (P = 0.43). Neutered cats had a greater prevalence of URD (39%, n = 57) than intact cats (26%, n = 27) (P = 0.03). When all pathogens were considered together, the risk of developing URD was 2.6× greater for carriers (53%, n = 47) than for non-carriers (23%, n = 37) (P < 0.002). All FHV-1 carriers (100%, n = 5) developed URD compared to cats without subclinical infections (28%, n = 51) (P < 0.002), whereas the risk was not significantly greater (P = 0.46) for FCV carriers (43%, n = 7). Although prevalence of *M. felis* shedding (21%, n = 54) was greater than for all other pathogens combined, the risk of developing URD was not significantly greater for those cats [odds ratio (OR) = 1.6] (P = 0.29). The sample

of cats with subclinical *B. bronchiseptica* infection upon admission was small (n = 6). However, these cats were significantly more likely to develop URD than were non-carriers (n = 181) (OR = 12.6; P = 0.01).

Cumulative risk of developing URD over time

Cats in the study were at the shelter from 2 to 191 d. The median length of stay before cats exhibited clinical signs of URD, were adopted, redeemed, or euthanized was 14 d. Median times to URD for carriers of FHV-1 and FCV were 6 and 2 d, respectively, compared with 11 d for non-carriers of these 2 viruses. Median time to the development of URD for cats with subclinical B. bronchiseptica infection at intake was 8 d compared to 12 d for non-carriers. As shown in Table 4, there was no significant effect of gender or age class on the cumulative risk of developing URD over time. The cumulative risk of developing URD was significantly greater for strays than for OS cats [hazard ratio = 2.46 (1.48 to 4.09), P = 0.001]. The likelihood of developing URD was 17% greater for strays than for OS cats after 30 d (Figure 1). Similarly, the cumulative risk of developing URD was greater for neutered than for intact animals [hazard ratio = 0.43 (0.23 to 0.78), P = 0.01]. The likelihood for onset of clinical URD was 9% higher for neutered than for intact cats by day 30 (Figure 2). Cats with subclinical infections had the greatest cumulative risk for URD [hazard ratio = 2.39 (1.44 to 3.99), P < 0.001] compared with non-carriers. By day 7, 29% of carriers were at risk of developing URD compared to 2.7% for non-carrier cats. By day 14, the risk had increased to 46% for carriers compared with 13% for non-carriers. This trend continued into week 3 with 55% of carriers versus 28% of non-carriers, and beyond 30 d with 76% of carriers versus 41% of non-carriers (Figure 3).

Discussion

The primary objective of this study was to determine the risk factors associated with subclinical upper respiratory infections in cats entering a Canadian shelter and those associated with



Figure 1. The effect of source of cat on cumulative probability of developing clinical URD over time.



Figure 3. The effect of carrier status of cat on cumulative probability of developing clinical URD over time.

subsequent onset of clinical URD. The 2.8% shedding rate for FCV in cats entering this shelter was lower than the prevalence of 11% reported in a similar survey in a California shelter (25). Kittens are known to be particularly vulnerable to FCV (26), but our study showed no effect of age on FCV prevalence. In concordance with findings by Pedersen et al (25), FCV infection did not increase over time (1% increase by day 10). In this study, cats were housed singly, thereby minimizing cat-cat transmission of the virus.

Shedding of FHV-1 was also low upon admission (2%). Although shedding of FHV-1 increased over time (8%), prevalence overall was much lower than that reported in a study by Pedersen et al (25) (> 50% after 1 wk). This pathogen may be a particular risk for shelter cats, as latent infections can be reactivated in response to stress and cause recrudescent clinical disease (27). *Mycoplasma felis* are normal commensal organisms of the upper respiratory tract, but some strains have been implicated in clinical URD in both household (28) and shelter cats (\geq 47%) (29,30). Our findings indicate a high prevalence of *M. felis* infection upon admission (21%) and showed the largest increase over time (10%) in cats that remained healthy.

Overall, the prevalence of clinical URD in this Canadian shelter (34%) was similar to rates reported in northeastern US shelters (33%) (5) and lower than rates reported in Californian shelters (55%) (14). Carriers (28%), particularly those with FHV



Figure 2. The effect of sterilization status of cat on cumulative probability of developing clinical URD over time.

or B. bronchiseptica infections, were at increased risk for onset of clinical URD. Only 5 cats were FHV-1 positive at intake; however, all developed URD. Similarly, of the 6 cats positive for B. bronchiseptica at intake, all but one developed URD. Although co-infections are common with B. bronchiseptica, this bacterium alone is capable of inducing respiratory disease (31), which was the case in this study. In a UK study, 19% of cats with URD were positive for B. bronchiseptica alone (32). However, an Italian study found more cases of co-infection (42 cases) compared with this bacterium alone (11 cases) (18). In accordance with Bannasch and Foley (14), M. felis was the most prevalent pathogen but was not significantly implicated in the development of URD. Similarly, C. felis is a common pathogen isolated from cats with confirmed conjunctivitis (33); however, it was not prevalent in this shelter. We concur with other authors (34) that this bacterium may not be an important risk factor for shelter cats. The H1N1 influenza virus can be transmitted naturally from humans to cats (35), and can be used to induce experimental infection in cats (36). Although this study was conducted during the human H1N1 pandemic of 2009, no cases were identified nor have any cases been reported in other shelters to date.

Overall, the only infectious agents with significant risk for onset of URD were FHV-1 and *B. bronchiseptica*. A recent study reported no significant difference in prevalence of URD between cats that were PCR positive and cats that were POCR negative for these organisms (37). These authors concluded that URD cannot be controlled by segregation of symptomatic animals due to a lack of strong correlation between subclinical infections and onset of URD. Rather, they recommended similar biosecurity protocols and stress management practices for all cats.

In addition to examination of carrier state as a risk factor, the multivariate analysis included age, gender, sterilization status, and source. Several authors agree that gender is not an important risk factor (14,19), although 1 study identified adult females as a low risk group (5). In our observations, there was no difference between male and female cats; however, neutered cats were at greater risk for URD. Edwards et al (20) found a similar trend, concluding that because most neutered cats were also owner-surrendered, they had likely not been exposed to URD pathogens and were therefore more vulnerable. In our study, more seniors than adults were neutered, which may explain the association, in accordance with findings that age represents a significant risk factor for the development of clinical URD, with the very young and old being most vulnerable to disease (5,15,20).

In this study, age was not a significant factor; however, inclusion in the study was restricted to cats older than 6 mo and juveniles were poorly represented (n = 50), which may account for the lack of significance. Although shelter staff received training to estimate the age of cats, exact age could not be determined for stray cats which may have introduced some bias. The finding that stray cats were more susceptible to clinical URD than owner-surrendered cats agrees with other authors (38) and may have been influenced by the urban setting (16). According to these authors, the high density of strays in urban settings increases the risk of contracting FCV and FHV-1 infections through social contact between cats (i.e., oral and nasal contact with secretions of infected cats). The most significant risk factor for developing clinical URD in this study was time spent at the shelter. Most authors agree that the risk of developing clinical URD increases with time spent in the shelter (5,25). However, 1 study reported a decline in clinical URD over time, with most signs occurring within 50 d of admission (20). It concluded that cats still at the shelter beyond 50 d were probably resistant to infection. Dinnage et al (5) cautioned against such an interpretation because the number of cats remaining in studies usually decreases over time; therefore, the cumulative probability may be increasingly imprecise.

The prevalences of subclinical infections and clinical URD were low in this shelter, which may be related to its policy of vaccination upon admission (39), placement of cats in quarantine upon admission (8), and good biosecurity (15,30). Although the practice is controversial, the immediate transfer of cats to an isolation ward upon onset of clinical signs is believed by some authors to reduce prevalence of disease (3,20). Others have suggested that the stress of moving to another cage may complicate disease (25) and increase severity (40,41). Finally, density of the population in a shelter is known to influence rate of transmission (42). This shelter had a capacity of 120 cats with several separate housing areas which may have contributed to a lower rate of viral reactivation and transmission. Furthermore, cats in this study (50%) were concurrently taking part in a stress management study which may also have contributed to reduced onset of disease.

This is one of the first studies in Canada to determine the infection status in cats on admission to an animal shelter and risk factors associated with clinical URD over time. Except for *M. felis,* prevalence of subclinical infections and subsequent spread of pathogen were less than that observed in shelters from other countries. However, risk factors for URD, such as FHV-1 and *B. bronchiseptica* infections and stray status, were in accordance with findings in other countries.

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