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Identification of pathogens in respiratory samples of shelter cats in New York State

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Abstract

Objectives

Upper respiratory tract disease (URTD) is common across feline populations. Feline coronavirus (FCoV) frequently circulates widely and has occasionally been noted to have a potential role in respiratory disease. The aim of this cross-sectional pilot study was to investigate common respiratory pathogens and FCoV in shelter cats.

Methods

Cats were enrolled at two animal shelters in New York state between November 2018 and March 2020 and considered either clinical for URTD or apparently healthy. Respiratory samples were submitted to the Cornell Animal Health Diagnostic Center for routine upper respiratory diagnostic testing. Additional qRT-PCR was performed on respiratory and fecal samples to investigate the presence of FCoV.

Results

Five pathogens were identified in this population: *Bordetella*, feline calicivirus (FCV), *M. felis*, panleukopenia, and pneumovirus. FCV was the only pathogen associated with URTD signs. Pneumovirus was identified in two cats. FCoV was present in respiratory samples from one cat, who later developed gastrointestinal disease. Fecal shedding of FCoV was observed in 30% of URTD cases, compared to 11% of cats without URTD, but was not statistically significant.

Conclusions and relevance

Common respiratory pathogens were identified in cats with and without URTD. One cat tested positive for FCoV in a respiratory sample; FCoV shedding the feces was common, but not statistically significant, in cats with respiratory disease. Two cats were identified as shedding pneumovirus. The significance of pneumovirus to feline respiratory disease remains unknown and in further need of study.

Introduction

Feline upper respiratory tract disease (URTD) is commonly encountered in animal shelters and rescues (1,2). High URTD rates can lead to increased use of antibiotics, higher cost of veterinary care, longer shelter stay due to time spent in isolation wards, and greater barriers to providing enrichment. Feline coronavirus (FCoV) is also common in cat populations (9). Clinical presentations of FCoV infection can take two forms: a common, mild, self-limiting to inapparent diarrheal disease; or the rare, systemic, lethal disease, feline infectious peritonitis (FIP) (10–12). Wolfe and Griesemeir, who first suggested a viral etiology for FIP, noted that 2 of 16 cats with FIP presented with upper respiratory signs (13). Kittens with URTD signs may frequently be FCoV seropositive (14). Recently, our lab demonstrated FCoV in the nasal cavity of a cat who died from FIP (15). Though these reports support an association of FCoV with upper respiratory disease, FCoV is rarely considered a respiratory pathogen. The objective of this pilot study was to investigate the role of FCoV as a component of URTD in cats in two New York state shelters.

Materials and Methods

Feline sampling & initial processing

Samples were solicited from two shelters in New York State. Cats were diagnosed with “upper respiratory tract disease” by the shelter veterinarian, based on nasal and/or ocular discharge, chemosis, lethargy, oral ulcerations, fever, etc. Cats who lacked clinical respiratory signs were classified as healthy. Samples were obtained while anesthetized at the time of spay/neuter surgery, with the exception of any cases of URTD deemed too sick to undergo anesthesia. Sample collection occurred between November 2018 and March 2020. Because of the COVID-19 pandemic, further sampling was discontinued in March 2020. For each cat, basic signalment was provided by the shelter. Age was estimated by the shelter and then categorized by life stage, in accordance with published guidelines (17).

Samples included oropharyngeal, nasal and conjunctival swabs and fecal material. To obtain conjunctival swabs, the lower eyelid was gently pulled to expose the conjunctival sac before the swab was inserted. Oropharyngeal swabs were obtained in proximity to the tonsils. Nasal samples were either obtained through insertion into the nares or by swabbing nasal discharge. Fecal material was collected from litterboxes. All samples were stored at 4°C and shipped on ice to the laboratory. On delivery, approximately 2mL of sterile DMEM (no additives) per swab was added to the tube containing swabs from each site, vortexed for 15 seconds and incubated for 30 minutes at 4°C before aliquoting. A total of 400mg of feces were diluted in 1.2mL of sterile phosphate buffered saline (PBS) and vortexed for 15 seconds. Fecal samples were incubated for at least 2 hours, up to overnight, at 4°C. Diluted fecal samples were centrifuged at 14,000 RPM for 10 minutes and the supernatant was drawn off. All samples were stored at -20°C.

Respiratory pathogen detection

Respiratory pathogen testing was performed through the New York State Veterinary Diagnostic Laboratory/Cornell Animal Diagnostic Center (AHDC; Ithaca, NY). Specifically, feline herpesvirus type-1 (FHV-1), feline calicivirus (FCV), and feline panleukopenia virus were detected through viral isolation, and *Bordetella*, *Chlamydia*, influenza A virus, *Mycoplasma cynos*, *Mycoplasma felis*, pneumovirus and *Streptococcus zooepidemicus* were detected by PCR. SARS-CoV-2 was not among the pathogens tested. Results of the PCR tests were reported as not detected, low-, moderate-, or high-positive, but in this report were coded as “positive” or “not detected.”

FCoV detection

RNA extraction was performed using either MAX Express (Life Technologies, Grand Island, NY, USA) or E.Z.N.A.® Viral RNA Kit (Omega Bio Tek, Norcross, GA). Quantitative RT-PCR was performed using Ultraplex 1-Step Tough Mix 4X (Quantabio, Beverly, MA, USA), as previously described by Dye and colleagues (16).

Data analysis

Univariate analysis of continuous and ordinal variables (age, categorical age, and weight) were evaluated using logistic regression. For categorical demographic variables, (shelter location, sex, and breed) association with the disease outcome was evaluated via Fisher’s exact test using a two-sided alternative. A p-value of less than 0.05 was considered significant.

Ethical approval

All procedures were approved by the Cornell IACUC #2012-0116. Both animal shelters were made aware of the risks and benefits of this study, willingly participated, and gave permission to publish the results of the tested samples.

Results

Population demographics

A total of 39 cats were enrolled, of which 20 were cases of URTD and 19 free from URTD, at the time of sampling. Cats with upper respiratory disease were younger ($p < 0.05$) compared to the healthy group (Table 1). Weight was correlated with age ($\rho = 0.72$, p -value < 0.05) and cats with URTD tended to weigh less ($p < 0.05$). Individual shelter was also associated with disease status ($p < 0.05$). Sex and breed were not associated with disease.

Pathogen detection

Of the 11 pathogens on the diagnostic panel, 5 were detected: *Bordetella*, feline calicivirus, *M. felis*, panleukopenia, and pneumovirus (Table 2). Fifteen cats with URTD had detectable pathogens and nine were positive for more than two pathogens. The highest number of pathogens detected in a single cat with URTD was four. Calicivirus was significantly associated with respiratory disease status ($p=0.001$, odds ratio (OR): 20.27, 95% confidence interval for OR: 2.32-991.89). No other pathogens detected in respiratory swabs were significantly associated with URTD. Pneumovirus was identified in two cases; however, the association was

not statistically significant. Both of these cats were also positive for calicivirus and *Bordetella*. One of the cats was also positive for *Mycoplasma felis* and shedding FCoV in the feces. Thoracic radiographs in one of the pneumovirus positive cats indicated a diffuse interstitial/bronchiolar pattern.

One or two pathogens were detected in nine of the healthy cats. A low level of FCoV was identified in a pooled respiratory sample from one kitten in the apparently healthy group. On intake to the shelter, this kitten was bright, alert, responsive, and euhydrated with no ocular or nasal discharge. Post-neutering, on day three of shelter intake, vomiting and diarrhea were noted (no further testing was performed). Finally, the shedding of FCoV in fecal samples was assessed across disease statuses to determine whether an association existed with clinical URTD. FCoV was identified in 30% (6/20) of fecal samples from cats with URTD and 16% (3/19) of healthy cats, including the kitten that was positive via respiratory sample. These proportions were not significant.

Discussion

In this study, five pathogens were detected in respiratory samples from cats with URTD. FCV was the only pathogen significantly associated with URTD. Detection of FCV was performed via viral isolation, supporting the pathogenic role. The healthy animal that was positive for FCV may have been in an early stage of infection, but was not further followed. *Bordetella* was relatively common. The prevalence of *Bordetella* in shelters is thought to be variable. Persistent shedding of *B. bronchiseptica* has been experimentally observed in kittens (18). Here, 2 of 18 apparently healthy cats were *Bordetella* positive. *Mycoplasma felis* was commonly found in cats, regardless of disease status. The role of *M. felis* in URTD has been debated, though accumulating evidence has supported an association with disease, especially conjunctivitis (19). In this study, we detected pneumovirus in two cases of URTD, both of which were also positive for *Bordetella* and FCV. Since 2010, canine pneumovirus has been identified across several canine populations and associated with respiratory disease outbreaks (20–23). Pneumovirus has been isolated from other cats with respiratory disease and passage of a feline virus in mice has resulted in lung pathology and cytokine changes (7). More research, however, is needed in regards to pneumovirus in cats.

Surprisingly, we did not detect FHV-1. The small sample size, inclusion of only two shelters, and population constrained by those needing spay/neuter likely contributed to this observation. Additionally, the utilization of viral isolation, compared to PCR may have accounted for the lack of FHV-1 detection (25). Viral isolation is highly specific (27) and at the time of investigation was the primary assay offered through the AHDC as part of their feline respiratory panel. PCR is highly sensitive and a concern with this technique is the detection of FHV-1 DNA from recent vaccination (28). Though unlikely, sampling technique or time between sampling to viral isolation could have both impacted the ability to isolate FHV-1. It's also possible that time between shelter intake and sampling was short enough that stress from shelter entry had not yet contributed to FHV-1 recrudescence, or, less likely, that the cats in this young population had not yet been exposed to FHV-1.

Chlamydia, *Mycoplasma cynos*, *Streptococcus equi* sbsp *zooepidemicus*, and influenza A virus were not observed in this study. A low prevalence of *Chlamydia felis* has previously been observed in shelters (24), though in cats with conjunctivitis, the PCR prevalence has been over 50% (29). *Mycoplasma cynos* has previously been identified in one cat with conjunctivitis, though more research is required to understand its pathogenic potential (29). By comparison *Mycoplasma cynos* has been identified in samples from dogs with respiratory disease (30, 31). Influenza virus remains a relatively rare occurrence in American cats and has primarily been associated with outbreak situations or spillover events and was not expected to be found in this study (32–34). Lastly, *Streptococcus equi* sbsp *zooepidemicus* is associated with respiratory signs in addition to pharyngeal and meningial disease (4), and unsurprisingly, was not identified in this study.

The Whittaker laboratory previously reported on the presence of FCoV in the respiratory passages of a cat with FIP (15). While FCoV is unequivocally associated with enteric shedding and FIP, the potential for upper respiratory tract involvement has also been recognized (13, 35, 36). Although this study did not

demonstrate FCoV in any cats sampled with respiratory signs, we did observe FCoV in the respiratory samples from an initially healthy cat. This kitten subsequently developed gastrointestinal signs common for FCoV infection, and so the preclinical identification of FCoV in respiratory swabs may indicate an early entry point for the virus across the respiratory epithelium.

The small sample size and convenience sampling are both limitations of this study and the results here may not be representative of shelter or owned cat populations more broadly. In regards to FCoV, based on the estimates here, a sample size of approximately 140 cases and 140 controls would be better able to detect whether a statistically significant difference exists in regards to FCoV fecal shedding and upper respiratory disease status. Because of the COVID-19 pandemic, the decision to discontinue sampling was made in March 2020.

In this study, younger cats were more likely to be clinical, test positive for respiratory pathogens, and test positive for FCoV. Age is frequently a contributing or confounding factor in studies of infectious diseases. Older cats may have acquired immunity against the investigated pathogens, either through previous infection or vaccination, but exposure history was not available for such evaluation (and rarely is). Furthermore, environments in which a young cat is at risk for acquiring respiratory disease may be the same in which they are at risk for acquiring GI disease. The population of cats sampled in this study was constrained by our humane protocols which encouraged sampling at the time of spay/neuter surgery. This resulted in both the sick and healthy populations being younger than the general population of cats in these shelters and as a whole. Future studies of these pathogens may increase their yield by targeting younger cats; prevalence studies should strive to sample more evenly across cat age groups.

Finally, only two shelters participated in this study. Cats sampled in this study were selected by veterinarians and staff at each shelter at their convenience, not at random, and not blindly. Both of these shelters have staff veterinarians, which means they are potentially able to care for animals that are sicker than shelters without veterinarians on staff.

Conclusions

URTD remains a challenging disease complex to manage, especially in densely housed populations of cats. In this pilot study, we identified two cats with respiratory signs that were shedding pneumovirus. These cats were also positive for other pathogens, so it remains inconclusive whether pneumovirus contributed to their respiratory disease. Respiratory samples from a single cat without respiratory disease signs were positive for FCoV; following sampling, this cat developed gastrointestinal signs of disease. While we did not detect a statistically significant association between fecal shedding of FCoV and respiratory disease, the small sample size limited the power of this study.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ("best practice") of veterinary clinical care for the individual patient were always followed. Ethical approval from a committee was therefore not specifically required for publication. All procedures were approved by the Cornell IACUC #2012-0116.

Informed consent

Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies).

Table 1: Basic characteristics of cats with and without upper respiratory disease.

IQR is interquartile range. † p-value <0.05

	URTD cats (n =20)	Healthy (n = 19)
<i>Sex</i>		
Female –count (%)	9 (45)	10 (53)
Male – count (%)	11 (55)	9 (47)
<i>Median age in months (IQR) †</i>	3.12 (4.53)	24 (14.52)
<i>Categorical age†</i>		
Kitten (up to 6 months) – count (%)	15 (75)	1 (5)
Junior (7 months-2 years) - count (%)	4 (20)	14 (74)
Prime (3-6 years) - count (%)	1 (5)	3 (16)
Mature (7-10years) - count (%)	0 (0)	1 (5)
<i>Shelter†</i>		
A - count (%)	11 (55)	3 (16)
B - count (%)	9 (45)	16 (84)
<i>Breed</i>		
DSH - count (%)	16 (80)	15 (79)
Other/unknown (%)	4 (20)	4 (21)
<i>Median weight in lbs (IQR) †</i>	1.56 (0.94)	3.63 (1.43)

Table 2: Specific pathogen detection in respiratory swabs. Pathogens were detected via PCR, with the exception of FHV-1, panleukopenia and FCV, which were investigated via viral isolation. † $p < 0.05$, significant association with the outcome was assessed via two-sided Fisher's Exact test).

	URTD cats: n = 20	Healthy cats: n = 19
	Count (%)	Count (%)
<i>Bordetella</i>	7 (35)	2 (11)
Feline calicivirus†	11 (55)	1 (5)
<i>Chlamydia</i>	0 (0)	0 (0)
Feline coronavirus	0 (0)	1 (5)
Feline herpesvirus type 1	0 (0)	0 (0)
Influenza virus	0 (0)	0 (0)
<i>Mycoplasma cynos</i>	0 (0)	0 (0)
<i>Mycoplasma felis</i>	8 (40)	7 (37)
Panleukopenia	1 (5)	0 (0)
Pneumovirus	2 (10)	0 (0)
<i>Streptococcus zooepidemicus</i>	0 (0)	0 (0)

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