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Feline ocular and respiratory infections: a retrospective analysis of clinical cases submitted to Georgia Veterinary Diagnostic Laboratories (2012–2022)

David Neely[1*], Yung-Yi C Mosley[1,2*], Shauna Morris[3], Rebecca P Wilkes[4], Eman Anis[5], Hemant K Naikare[6] and Susan Sanchez[2]

Abstract

Objectives The objective of this study was to retrospectively assess the pathogens associated with feline ocular and respiratory diseases in routine diagnostic samples submitted to Georgia Veterinary Diagnostic Laboratories. Furthermore, pathogens detected by the respiratory PCR panel in the upper vs lower respiratory tract were compared (specimen separation at pharynx).

Methods Test records from feline ocular and respiratory PCR panels were collected from 2012 to 2022. While the ocular panel targets feline herpesvirus-1 (FHV), feline calicivirus (FCV) and *Chlamydia felis*, the respiratory panel covers FHV, FCV, *C felis*, *Mycoplasma* species, *Bordetella bronchiseptica* and influenza A virus.

Results In total, 120 and 453 submissions were made for the ocular and respiratory panels, with positivity rates of 49.2% (59/120) and 69.3% (314/453), respectively. Based on the available signalment, cats aged younger than 3 years made up 59.3% (32/54) and 47.3% (130/275) of positive cases, respectively. The top two findings by ocular and respiratory panels were single detection of FCV (28.8%, 17/59), FHV (25.4%, 15/59) and *Mycoplasma* species (36.9%, 116/314), co-detection of FCV + *Mycoplasma* species (20.4%, 64/314), respectively. The most common detection from samples of upper vs lower respiratory tract was *Mycoplasma* species (39.6%, 97/245) and FCV + *Mycoplasma* species (25%, 10/40).

Relevance and novel information FCV and FHV were the most frequent detections by the ocular panel, with *Mycoplasma* species and FCV + *Mycoplasma* species being the most frequent detections by the respiratory panel. Incorporating FCV and FHV in the vaccination regimen could help reduce the cases of feline ocular and respiratory diseases, especially for cats aged younger than 3 years. Veterinarians should consider *Mycoplasma* species when dealing with feline respiratory infections.

Keywords: FHV; FCV; Georgia; *Mycoplasma*; ocular; PCR; respiratory; retrospective

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[1]Tifton Veterinary Diagnostic & Investigation Laboratory (TVDIL), University of Georgia, Tifton, GA, USA
[2]Department of Infectious Diseases, University of Georgia, Athens, GA, USA
[3]Athens Veterinary Diagnostic Laboratory (AVDL), University of Georgia, Athens, GA, USA
[4]Animal Disease Diagnostic Laboratory, Purdue University, West Lafayette, IN, USA
[5]Department of Pathobiology, University of Pennsylvania, School of Veterinary Medicine, New Bolton Center, Kennett Square, PA, USA
[6]Minnesota Veterinary Diagnostic Laboratory, Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

*David Neely and Yung-Yi C Mosley contributed equally to this work

Corresponding author:
Yung-Yi C Mosley BVM, MS, PhD, DACVM, Tifton Veterinary Diagnostic & Investigation Laboratory (TVDIL), University of Georgia, 43 Brighton Rd, Tifton, GA 31793, USA
Email: yymosley@uga.edu

Introduction

Respiratory illness is a common reason for cats to visit a veterinary clinic[1] and euthanasia in some shelters.[2] Infectious agents are often the cause of feline respiratory diseases. Two viruses, feline calicivirus (FCV) and feline herpesvirus-1 (FHV), both cause coughing, sneezing and ocular/nasal discharge, but FCV can also cause gingivitis/stomatitis or oral ulcers.[3–5] Bacterial organisms such as *Chlamydia felis*, *Bordetella bronchiseptica* and *Mycoplasma* species can cause ocular/nasal discharge, sneezing and conjunctivitis.[3,6–10] While *C felis* is more likely to cause ocular signs than respiratory illness,[6] *B bronchiseptica* causes bronchopneumonia and cough, although cough is less common in cats than dogs.[3,9,10] A highly pathogenic influenza A virus such as H5N1 can induce high fever, ataxia, circling and high mortality,[11] and a low pathogenic influenza virus originating from humans, dogs or birds causes cough, sneezing and ocular/nasal discharge with more serious signs often attributed to secondary bacterial infections.[12] Further testing is often required to differentiate which pathogen is present in feline ocular and respiratory infections. Diagnosis and treatment are often presumed, as additional tests can be costly and time-consuming.

PCR testing has largely replaced bacterial culture and virus isolation due to higher sensitivity and quicker turnaround times.[13] Georgia Veterinary Diagnostic Laboratories (GVDLs) provide two options for PCR panels: the ocular panel targeting FHV, FCV and *C felis*, and respiratory panel detecting FHV, FCV, *C felis*, *Mycoplasma* species, *B bronchiseptica* and influenza A virus. This study aimed to summarize the findings of the ocular and respiratory panels from clinical cases submitted to GVDLs, including infectious agents detected in the upper and lower respiratory tracts.

Materials and methods

Source of samples

The 2012–2022 ocular and respiratory PCR data were retrieved from VetView, the GVDLs' (Athens and Tifton) Laboratory Information Management System. Specimens such as conjunctival/nasal/pharyngeal swabs, transtracheal wash, bronchoalveolar lavage and nasal/lung tissue were submitted from veterinarians, the veterinary teaching hospital of University of Georgia or in-house pathology/necropsy services. Submission sheets were sent with samples containing signalment, specimen type with physical location and the requested test, either ocular or respiratory PCR panel. Information about history, treatment and housing situation is often left blank on submission sheets.

Real-time PCRs

The real-time PCR methods have been previously described for the targets in the ocular and respiratory panels including FHV,[14] FCV,[15,16] *C felis*[14]/*Chlamydia* species[17] and *Mycoplasma* species,[18] *B bronchiseptica*[19] and influenza A virus.[20] Primer and probe sequences are listed in Table 1. C_t values lower than 35 or 40 are defined as positive detection. All PCR methods were conducted with proper controls to the standards of the American Association of Veterinary Laboratory Diagnosticians.

Data analysis

Excel spreadsheets (Microsoft) were used to analyze pathogen detections and locations in each age group (defined according to the American Association of Feline Practitioners and American Animal Hospital Association charts, with ages rounded to the nearest group) (Table 2). Specimens taken from below the pharynx are defined as lower respiratory samples, such as transtracheal wash and lung tissue. Pathogen detection between age groups was compared by Fisher's exact test and correlation matrix by Spearman's correlation in Prism, version 10 (GraphPad Software).

Results

Between 2012 and 2022, 120 submissions were received for ocular panel. Ocular sites such as conjunctival/corneal swab and ocular fluid comprised 56.7% (68/120) of the specimens and 17.5% (21/120) were from mixed locations including an ocular site (eg, nasal/conjunctival swab). The remaining 31 specimens were taken from upper respiratory areas such as nasal/oral or undefined sites.

The most common detection was FCV (28.8% [17/59]), followed by FHV (25.4% [15/59]) and *C felis* (20.3% [12/59]). Fifteen cases (25.4%) had more than one pathogen detected, including FCV + FHV (10.2% [6/59]), FCV + *C felis* (6.8% [4/59]), FHV + *C felis* (5.1% [3/59]) and FCV + FHV + *C felis* (3.4% [2/59]) (Figure 1). Pathogens were analyzed to examine if certain agents tended to be detected together. There was no significant correlation between the pathogens ($P > 0.05$).

Cats aged younger than 3 years made up 59.3% (32/54) of the cases (Table 2). While 47.1% (8/17) of FCV detections were from non-ocular sites (such as nasal/oral/lung), most single detections of *C felis* (91.7%, 11/12) and FHV (80.0%, 12/15) were from ocular specimens.

Between 2012 and 2022, 453 submissions were received for respiratory panel. Overall, the panel showed 30.7% (139/453), 14.6% (66/453), 2.4% (11/453), 51.9% (235/453), 3.5% (16/453) and 0.2% (1/453) positivity rate for FCV, FHV, *C felis*, *Mycoplasma* species, *B bronchiseptica* and influenza A virus, respectively. Most specimens (82.3%, 373/453) were taken from respiratory sites such as nasal/oral/tracheal swab, nasal flush/tissue and lung tissue, 3.09% (14/453) of samples were from ocular sites and 5.96% (27/453) were from mixed locations including an ocular site (eg, oral/nasal/conjunctival

Table 1 Primer/probe used in the ocular and respiratory panels at Georgia Veterinary Diagnostic Laboratories

Athens respiratory and ocular panel		Reference
FHV		14
FW	5′-GGA CAG CAT AAA AGC GAT TG-3′	
Rev	5′-AAC GTG AAC AAC GAC GCA G-3′	
probe	5′-AAT TCC AGC CCG GAG CCT CAA T-3′	
FCV		15
FW	5′-GTT GGA TGA ACT ACC CGC CAA TC-3′	
Rev	5′-CAT ATG CGG CTC TGA TGG CTT GAA ACT G-3′	
Probe	5′-TCG GTG TTT GAT TTG GCC TG-3′	
Chlamydia felis		14
FW	5′-GAA CTG CAA GCA ACA CCA CTG-3′	
Rev	5′-CCA TTC GGC ATC TTG AAG ATG-3′	
Probe	5′-CGC TGC CGA CAG ATC AAA TTT TGC C-3′	
Mycoplasma species		18
FW	5′-GGG AGC AAA CAG GAT TAG ATA CCC T-3′	
Rev	5′-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3′	
Bordetella bronchiseptica		19
FW	5′-AGG CTC CCA AGA GAG AAA GGC TT-3′	
Rev	5′-TGG CGC CTG CCC TAT C-3′	
Influenza A virus		20
FW primer	5′-AGA TGA GTC TTC TAA CCG AGG TCG-3′	
Rev 2002	5′-TGC AAA AAC ATC TTC AAG TCT CTG-3′	
Rev 2009	5′-TGC AAA GAC ACT TTC CAG TCT CTG-3′	
Probe	5′-TCA GGC CCC CTC AAA GCC GA-3′	
Tifton ocular panel (same as Athens except for the following)		
FCV		16
FW	5′-GTA AAA GAA ATT TGA GAC AAT-3′	
Rev	5′-TAC TGA AGW TCG CGY CT-3′′	
Probe	5′-CAA ACT CTG AGC TTC GTG CTT AAA-3′	
Chlamydia species		17
FW	5′-CTG AAA CCA GTA GCT TAT AAG CGG T-3′	
Rev	5′-ACC TCG CCG TTT AAC TTA ACT CC-3′	
Probe	5′-CTC ATC ATG CAA AAG GCA CGC C-3′	

FCV = feline calicivirus; FHV = feline herpesvirus-1; FW = forward; Rev = reverse

Table 2 Age groups and the detection numbers by ocular and respiratory panels

Stage	Age	Number (%) of detection by ocular panel (54/59)*	Number (%) of detection by respiratory panel (275/314)*
Kitten	≤6 months	17/54 (31.5%)	60/275 (21.8%)
Junior	7 months to 2 years	15/54 (27.8%)	70/275 (25.5%)
Prime	3 to 6 years	6/54 (11.1%)	52/275 (18.9%)
Mature	7 to 10 years	9/54 (16.7%)	51/275 (18.5%)
Senior	11 to 14 years	6/54 (11.1%)	22/275 (8.0%)
Geriatric	≥15 years	1/54 (1.9%)	20/275 (7.3%)

*The portion of the positive detections with age recorded on the submission sheet.

swab). The remaining 8.61% (39/453) specimens were taken from other or undefined sites.

The most common single detection was *Mycoplasma* species (36.9%, 116/314), followed by FCV (13.7%, 43/314) and FHV (7.6%, 24/314) (Figure 2). Multipathogens comprised 127 cases (40.4%), including FCV + *Mycoplasma* species (20.4%, 64/314), FHV + *Mycoplasma* species (5.41% 17/314) and FHV + FCV + *Mycoplasma* species (4.78%, 15/314) (Figure 2). No cases tested positive for more than four pathogens (Figure 2). A significant correlation was

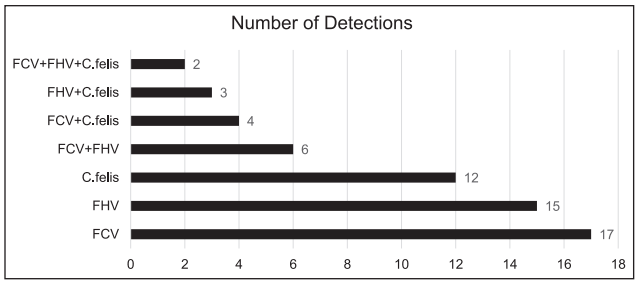

Figure 1 Number of detections by feline ocular PCR panel at Georgia Veterinary Diagnostic Laboratories from 2012 to 2022. Panel targets include feline herpesvirus-1 (FHV), feline calicivirus (FCV) and *Chlamydia felis*/*Chlamydia* species In total, 59 cases had at least one organism detected from 120 submissions (49.2% positivity rate)

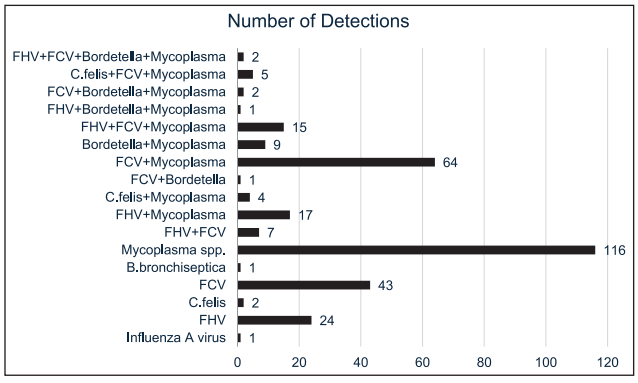

Figure 2 Number of detections by feline respiratory PCR panel at Georgia Veterinary Diagnostic Laboratories from 2012 to 2022. Panel targets include feline herpesvirus-1 (FHV), feline calicivirus (FCV), *Chlamydia felis*, *Bordetella bronchiseptica*, *Mycoplasma* species and influenza A virus. In total, 314 cases had at least one organism detected from 453 submissions (69.3% positivity rate)

identified between co-detection of FCV + *Mycoplasma* species ($P = 0.01$), *C felis* + *Mycoplasma* species ($P = 0.044$) and *Bordetella* species + *Mycoplasma* species ($P = 0.004$) (Figure 3).

Age distributions showed that most infections were among junior animals, followed by kittens and prime (25.5%, 21.8% and 18.9%, respectively) (Table 2). The most common pathogens found in cats aged 10 years and under were *Mycoplasma* species, FCV + *Mycoplasma* species and FCV (Figure 4). For cats younger than 7 years old (kitten + junior + prime), FHV detections were significantly less than those of other age groups ($P = 0.0099$), while FCV + *Mycoplasma* was significantly less in senior and geriatric cats than in younger age groups ($P = 0.042$) (Figure 4).

While most detections were from respiratory specimens (81.8%, 257/314), the majority of the triple detection of *C felis* + FCV + *Mycoplasma* species (80%, 4/5) were from mixed sites including an ocular site (eg, oral/nasal/conjunctival swab). The only positive specimen

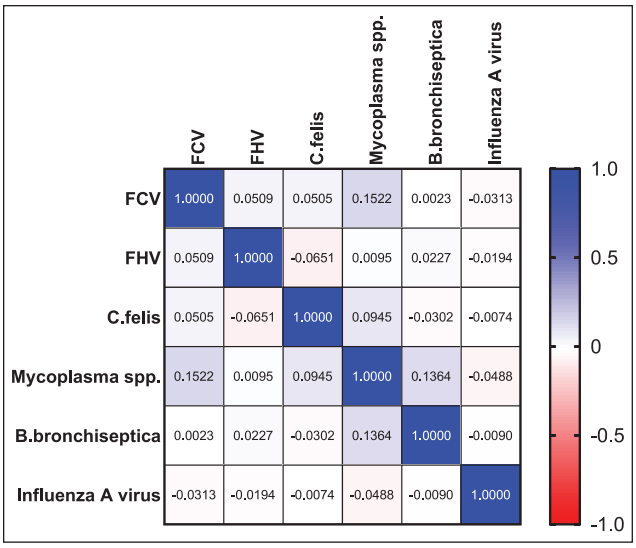

Figure 3 Pathogen detection correlation matrix by feline respiratory PCR panel. Number 0 or 1 was assigned for negative or positive results, respectively, for conducting the correlation coefficient analysis. Spearman's *r* is shown for each pair of pathogens. Four pathogens showed significant correlation of co-detection including FCV + *Mycoplasma* species ($P = 0.001$), *Chlamydia felis* + *Mycoplasma* species ($P = 0.044$) and *Bordetella* species + *Mycoplasma* species ($P = 0.004$). FCV = feline calicivirus; FHV = feline herpesvirus-1

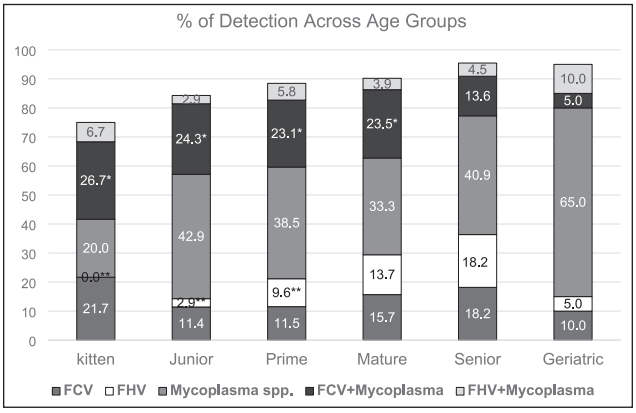

Figure 4 Pathogen detection percentage by feline respiratory PCR panel. Of the 314 positive cases, 275 had age provided. The kitten, junior, prime and mature age groups all had feline calicivirus (FCV), *Mycoplasma* species and FCV + *Mycoplasma* species as the most common pathogens. The senior age group had *Mycoplasma* species, feline herpesvirus-1 (FHV) and FCV as the most common pathogens. The geriatric age group had *Mycoplasma* species and a tie between FHV + *Mycoplasma* species and FCV. *Coinfection of FCV + *Mycoplasma* species is significantly less common in senior and geriatric cats than in younger cats ($P = 0.042$); **single FHV infection is significantly less common in cats younger than 7 years (kitten + junior + prime) than in cats older than 7 years (mature + senior + geriatric) ($P = 0.0099$)

identified for influenza A virus was lung tissue. Based on the recorded physical locations, specimens from the respiratory panel were further divided into upper vs

Table 3 Detection numbers of specimens taken from upper vs lower respiratory tract* by the respiratory panel

	Sample #	Positive #	Single positive	Multiple positives
Upper	351	245 (69.8%)	149 (60.8%)	96 (39.2%)
Lower	61	40 (65.6%)	21 (52.5%)	19 (47.5%)

*Specimen separation at pharynx: specimens taken from below the pharynx are defined as lower respiratory samples (eg, transtracheal wash and lung tissue); examples of upper respiratory specimen include conjunctival/oropharyngeal/nasal swabs and nasal tissues/flushes

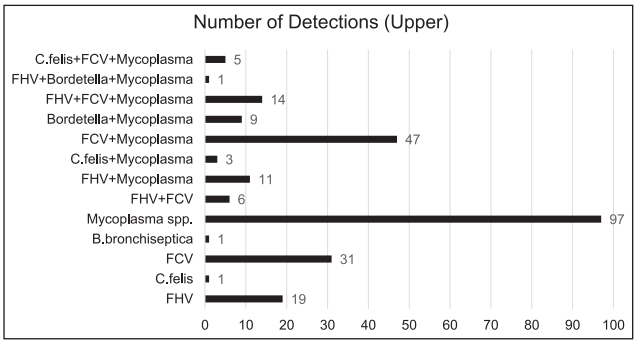

Figure 5 Number of detections from the upper respiratory tract by feline respiratory PCR panel. Among specimens taken from upper respiratory tract, 69.8% (245/351) tested positive with the three most common pathogens being *Mycoplasma* species, FCV + *Mycoplasma* species and FCV. FCV = feline calicivirus; FHV = feline herpesvirus-1

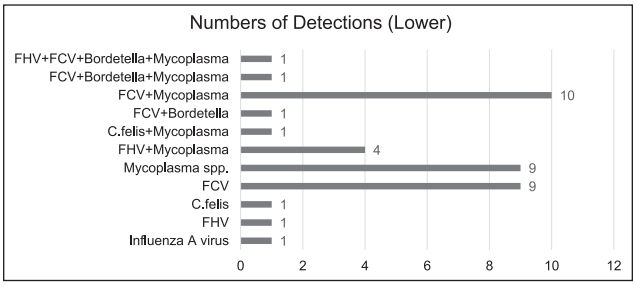

Figure 6 Number of detections from the lower respiratory tract by feline respiratory PCR panel. Among specimens taken from lower respiratory tract, 65.6% (40/61) tested positive with the three most common pathogens being FCV + *Mycoplasma*, *Mycoplasma* species and FCV. FCV = feline calicivirus; FHV = feline herpesvirus-1

lower respiratory tract. Positivity rates were slightly higher in upper respiratory samples (Table 3). While single pathogen cases were higher in upper respiratory samples, multipathogen detections were higher in lower respiratory samples (Table 3). The top three detections were the same for upper vs lower respiratory tract but in different order: *Mycoplasma* species (39.6%, 97/245), FCV + *Mycoplasma* species (19.2%, 47/245) and FCV (12.7%, 31/245) vs FCV + *Mycoplasma* species (25%, 10/40) and a tie between *Mycoplasma* species and FCV (22.5%, 9/40), respectively (Figures 5 and 6). While no case had four or more pathogens in the upper respiratory tract, one case of FHV + FCV + *Bordetella* species + *Mycoplasma* species was identified in the lower respiratory tract (lung tissue) (Figures 5 and 6).

Discussion

Prevalence studies for feline ocular and upper respiratory infections have been reported from the UK,[21] Germany,[22] Spain[23] and Australia,[24] as well as for shelter animals in Korea,[25] Canada[26] and the USA.[13,27,28] However, retrospective data have not been reported from a diagnostic laboratory in the USA. To give regional veterinarians a better idea of the pathogens detected in feline ocular and respiratory infections, we summarized the 2012–2022 results from GVDLs' PCR panels.

This study included samples submitted by veterinarians and was not exclusively obtained from shelter populations. Our findings are consistent with those investigating general populations, with FCV and *Mycoplasma* species being the two most detected pathogens.[23,24] On the other hand, studies focusing on shelter cats mostly found FHV or *Mycoplasma* species to be the most detected pathogen.[13,25–27]

Not all submissions for the ocular panel comprise only ocular specimens. While some veterinarians may have chosen the ocular panel over the respiratory panel for economic reasons, others may have taken swabs from multiple respiratory sites to increase the chance of pathogen detection. After all, respiratory tract infections often involve signs in both ocular and respiratory systems.[3–10,12]

Interestingly, *Mycoplasma* species comprise most detections of both upper and lower respiratory submissions. Although, *Mycoplasma* species are considered to be a commensal of the upper airway and conjunctiva of cats, a weak but significant association was found between *Mycoplasma* species and upper respiratory tract disease in a meta-analysis study.[29] While it is not conclusive that *Mycoplasma* species are a primary pathogen of the upper respiratory tract in the current study, the fact that it is the only detected agent in most upper respiratory disease cases does provide some evidence to support this argument. Yet, the detection of a pathogen does not mean it is solely responsible for causing the disease, as other pathogens could have been missed if early in the course or other etiologies such as asthma or neoplasia could also be involved predisposing to mycoplasmal infection.[8] *Mycoplasma* species detection was found to be significantly correlated between FCV, *C felis* and *B bronchiseptica* (Figure 3), suggesting the role of secondary infection in those cases.

FCV was detected more often than FHV in the current study; however, FHV infection leads to latent infection after recovery, and thus, the prevalence of FHV could be underestimated. While 23.9% (75/314) cases submitted for the respiratory panel had viral-only detections, about 76.1% (239/314) were bacterial-only or had bacterial components, suggesting that empirical treatment with antibiotics may have merit. *B bronchiseptica* and influenza A virus were detected in very few cases, suggesting they are not main contributors to feline respiratory cases submitted to our laboratories in Georgia.

Kittens and junior cats (<3 years old) seemed to make up most cases received in both diagnostic laboratories and had the most mixed infections. Although low in number, mature and senior cats had a larger percentage of FHV infections (Figure 4), possibly due to latent FHV recrudescing in older animals. *Mycoplasma* species had the most frequent detection in all age groups, except in kittens where it was the second most common.

Due to the limitation of the data source, we did not have complete histories for most submitted cases. Lack of information on treatment, housing conditions and clinical signs hampered our ability to draw stronger conclusions from our data. Nevertheless, it is fair to assume that animals submitted for the ocular and respiratory PCR panels were showing signs consistent with infection of either ocular, respiratory or both. Given this, it is reasonable to conclude that positive detections by the PCR panels are likely to be at least contributing to the disease state. Another limitation of the current study is that PCR results cannot rule out pathogens not included in the panel such as *Staphylococcus* and *Streptococcus* species.

Conclusions

FCV and FHV were the most frequent detections by the ocular panel, with *Mycoplasma* species and FCV being the most frequent detections by the respiratory PCR panel. As many cases involved FCV and/or FHV, incorporating FCV and FHV in vaccination regimens could help reduce the number of cases and/or lessen the clinical signs. *Mycoplasma* species were the major pathogen detected in both upper and lower respiratory tracts. Despite its controversial role as a primary pathogen, there is value in considering antibiotic treatment for *Mycoplasma* species in feline respiratory cases.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

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, tissues and samples) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore, additional informed consent for publication was not required.

ORCID iD David Neely https://orcid.org/0009-0001-6826-6644
Yung-Yi C Mosley https://orcid.org/0000-0003-4413-6332
Shauna Morris https://orcid.org/0009-0005-0079-4407
Rebecca Wilkes https://orcid.org/0000-0003-4846-0439
Eman Anis https://orcid.org/0000-0002-2084-1902
Hemant K Naikare https://orcid.org/0000-0003-3221-3222
Susan Sanchez https://orcid.org/0000-0002-2438-5983

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