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ORIGINAL ARTICLE

Prevalence study of respiratory pathogens in Korean cats using real-time polymerase chain reaction

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Pathogens such as feline herpesvirus, feline calicivirus, *Bordetella bronchiseptica*, *Chlamydia felis*, *Mycoplasma felis* and *Pasteurella multocida* usually cause feline upper respiratory tract disease (URTD). Real-time PCR was used to analyze the detection and prevalence of the most common respiratory pathogens in cats with (n=69) and without respiratory signs (n=31). Pathogens were detected in 53 cats, divided into 37 (69.8%) with a single pathogen, 15 (28.3%) with two pathogens, and 1 (1.9%) with three pathogens. *M. felis* had the highest detection rate in 29 (42.0%) cats, *P. multocida* was detected in 18 (26.1%), FHV in 10 (14.5%), FCV in 7 (10.1%), *B. bronchiseptica* in 3 (4.3%), and *C. felis* in 2 (2.9%). *M. felis* was the most frequently detected pathogen in cats living outdoors without vaccination. Of the 37 cats infected with single pathogen, nasal discharge was observed in 13 (35.1%), ocular signs in 6 (16.2%), drooling in 5 (13.5%), dyspnea in 3 (8.1%), and asymptomatic in 10 (27.0%). In 51 outdoor and 49 indoor cats, pathogens were detected in 35 (68.6%) and 18 (36.7%) cats, respectively. Of the 29 cats infected with *M. felis*, 22 (75.9%) showed respiratory signs, and 7 (24.1%) were healthy. In the age of the 53 positive cats, 10 (18.9%) were under the age of 1 year, 26 (49.1%) were aged 1～3 years, and 17 (32.1%) were aged 3 years or older. Although the number of cats in the study was small, the results can provide valuable data on the prevalence of URTD in Korean cats.

Key Words: Korean cat, Respiratory system, Pathogen, Real-time PCR, Prevalence study

INTRODUCTION

Feline upper respiratory tract disease (URTD) includes a wide range of clinical signs and is not a specific disease. It is defined as a syndrome with clinical signs, such as nasal and ocular exudates, sneezing, conjunctivitis, cough, fever, lack of energy, and loss of appetite. The causes of feline URTD are divided into infectious and non-infectious causes. First, as for infectious causes, viruses such as feline herpesvirus (FHV) and feline calicivirus (FCV); primary bacteria such as *Bordetella bronchiseptica* (*B. bronchiseptica*), *Chlamydia felis* (*C. felis*), and *Mycoplasma felis* (*M. felis*); and secondary bacteria such as *Pasteurella multocida* (*P. multocida*) are suggested as major pathogens (Holst et al, 2010; Sykes, 2014; Litster et al, 2015).

M. felis may be part of the normal bacterial flora in the feline upper respiratory tract but is mainly found in cats with respiratory signs or healthy cats living with infected cats. Recently, it has been considered a major pathogen in feline URTD (Holst et al, 2010; Hong et al, 2015; Lobova et al, 2019). However, a vaccine against *M. felis* infection has not yet been developed. Therefore, cats infected with *M. felis* are managed with adjuvant therapy to improve immunity along with long-term antibiotic administration (Lappin et al, 2017).

P. multocida is a gram-negative bacterium classified into five serogroups (A, B, D, E, and F) based on capsule composition and somatic serotype (Kuhnert and Christensen, 2008). *P. multocida* may be part of the normal

bacterial flora in cats, but it is also a causative pathogen of pneumonia, conjunctivitis, rhinitis, gingivostomatitis, abscesses, and osteonecrosis in cats (Ewers et al, 2006). It may also cause secondary infections in the lower respiratory tract and is associated with spinal empyema and meningoencephalomyelitis (Lloret et al, 2013).

Herpesvirus infection, also known as feline viral rhinotracheitis (FVR), is caused by the feline herpesvirus 1 (FHV-1). This species-specific virus infects individuals of all ages. FHV-1 is a major causative agent of URTD and conjunctivitis (Sykes et al, 1999; Cao et al, 2002; Helps et al, 2005; Litster et al, 2015; Maazi et al, 2016; Walter et al, 2020). Although no vaccine can completely protect against infection, clinical signs can be alleviated.

FCV is an enveloped small RNA virus belonging to the genus *Vesivirus* and the family *Caliciviridae* (Möstl et al, 2015). After replication in oral and respiratory tissues, FCV is excreted in saliva, feces, urine, and respiratory secretions and is transmitted through the air, oral cavity, and vectors. The clinical signs of FCV-infected cats can be acute, chronic, or asymptomatic, but when the cat is stressed, they develop clinical signs (Helps et al, 2005; Walter et al, 2020). Although there is no specific treatment for FCV, clinical signs can be alleviated through vaccination against FCV (Berger et al, 2015).

B. bronchiseptica is a gram-negative bacterium that inhabits the respiratory tract of mammals and is considered a major pathogen in domestic cats. Cats infected with *B. bronchiseptica* secrete the pathogen via oral and nasal secretions (Helps et al, 2005; Egberink et al, 2009; Litster et al, 2015; Lobova et al, 2019; Walter et al, 2020). Cats with respiratory diseases have a high risk of infection with *B. bronchiseptica*, which forms colonies on the ciliary epithelium of the host respiratory tract and causes a chronic infection. The main clinical signs are associated with a wide range of respiratory signs, ranging from mild signs such as fever, coughing, sneezing, ocular discharge, and lymphadenopathy to severe pneumonia with dyspnea, cyanosis, and death. *B. bronchiseptica* can be treated using antibiotics (Lappin et al, 2017).

C. felis is a gram-negative bacterium belonging to the family *Chlamydiaceae* and genus *Chlamydia*. It is transmitted through eye secretions upon close contact with an infected cat (Helps et al, 2005; Holst et al, 2010; Walter et al, 2020). Chlamydiosis usually have ocular signs, such as hyperemia, blepharospasm, and conjunctivitis, in kittens under 9 months of age. Cats with chlamydiosis are treated with antibiotics (Gruffydd-Jones et al, 2009; Lappin et al, 2017).

PCR tests for differential diagnosis of URTD have become common (Sykes et al, 1999; Kang and Park, 2008 Holst et al, 2010; Berger et al, 2015; Hong et al, 2015; Litster et al, 2015; Lobova et al, 2019; Walter et al, 2020), and are performed according to the degree of response to the primary diagnosis and treatment of the infection. However, some infectious agents, such as Mycoplasma, FHV, FCV, and *C. felis*, may be amplified even in individuals without clinical signs and may be detected in connection with vaccinations. If there are clinical signs of URTD and no vaccination history, the diagnosis of infectious diseases can be considered significant.

Therefore, the purpose of this study was 1) to analyze the most commonly infected pathogens in feline URTD, 2) to identify whether the infection is related to the living environment, vaccination, age, and clinical signs, and 3) to investigate single or complex infections in Korean cats.

MATERIALS AND METHODS

Target animal

This study was conducted on 100 cats who visited the Royal Animal Medical Center (Seoul, Korea) from 2019~2020. Sixty-nine cats showed respiratory disease signs, and 31 cats showed no clinical signs.

Sampling according to clinical signs

A cotton swab was inserted into the inner part of the eyelid to collect discharge from the eyes, deep inside of

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the nasal cavity for nasal exudate, or the larynx for laryngopharyngeal exudate. After collection, it was placed in a UTM container and stored at 4℃. Specimens were transferred to a diagnostic laboratory (Popanilab, Korea) within 24 h, and real-time polymerase chain reaction (real-time PCR) tests were performed.

Real-time polymerase chain reaction (real-time PCR)

The swab was immersed in a lysis solution, incubated for 10 min, and the DNA was extracted using Whatman binding plates on a Corbett X-Tractor platform (Qiagen, Düsseldorf, Germany). Nucleic acids were eluted in 150 μL PCR-grade nuclease-free water (Fisher Scientific, Pittsburgh, USA), of which 5 μL was used for subsequent real-time PCR amplification reactions. For FCV, 20 μL of total nucleic acid was reverse-transcribed into cDNA using random hexamer primers and SuperScript III (Invitrogen, Massachusetts, USA) in a final volume of 40 μL. Five microliters of diluted cDNA solution were used for real-time PCR using an FCV-specific assay.

The real-time PCR system utilized in this study is the Aria MX real-time PCR system (Agilent, Santa Clara, CA, USA), a fully integrated quantitative PCR amplification, detection, and data analysis system. The 15 real-time PCR test items included were FCV, influenza A/B, feline reovirus, FHV, *P. multocida*, *Moraxella catarrhalis* (*M. catarrhalis*), *Bartonella* spp., *C. felis*, *Aspergillus* spp., *M. felis*, *B. bronchispetica*, *Cryptococcus* spp., *Pneumocystis carinii* (*P. carinii*), *Histoplasma capsulatum* (*H. capsulatum*), and *Blastomyces dermatitidi* (*B. dermatitidi*). Target genes for respiratory pathogen detection using real-time PCR included genes from FCV (ORF1), Influenza A/B (M/NP), feline reovirus (L3), FHV-1 (glycoprotein B, gB), *P. multocida* (toxA), *M. catarrhalis* (copB), *Bartonella* spp. (intergenic transcribed spacer, ITS), *C. felis* (outer membrane protein A, OmpA), *Aspergillus* spp. (ITS1), *M. felis* (simple sequence repeats, ssr), *B. bronchiseptica* (Filamentous hemagglutinin gene, FhaB), *Cryptococcus* spp. (cytochrome b, cyt b), *P. carinii* (DHFR), *H. capsulatum* (mtSSU), *B. dermatitidis* (BAD1) (Table 1).

To determine the analytical sensitivity (the lower limit of detection) for individual target genes for enteric pathogens, serial diluents (10^5 to 1 copies/reaction) of synthetic DNAs or transcript RNAs for enteric pathogens were analyzed using qPCR or qRT-PCR. The lower limit of detection was defined as the lowest concentration that was detected in ≥95% of the replicates (Gong et al, 2018).

Data analysis

Cats used in this study were classified according to gender, age, and breed. Age was classified as less than 1 year old, 1 to 3 years old, and over 3 years old. Gender was divided into male and female, including whether neutering was performed. The living environment was classified as an indoor cat if living indoors with a guardian, and an outdoor cat if not. In addition, the overall prevalence and positive rate, and the frequency of single and multiple infections were distinguished, and the ratio of cats with and without URTD sings was additionally analyzed.

RESULTS

Of the 100 cats that participated in this study, 46 were neutered males, 48 were females, and the sex of 6 was not identified. In terms of the living environment, 49 lived indoors (owned), and 51 lived outdoors (stray). The study included 15 breeds. Those were: Abyssinian (2 cats), American short hair (1 cat), Bengal (3 cats), British Shorthair (1 cat), Korean short hair (73 cats), Maine coon (2 cats), Munchkin (2 cats), Norwegian forest (3 cats), Persian (2 cats), Ragdoll (1 cat), Scottish fold (4 cats), Siamese (2 cats), Singapura (1 cat), Sphynx (1 cat), and Turkish angora (2 cats).

Pathogens were detected in 53 of 100 cats using real-time PCR tests. A single pathogen was detected in 37 (69.8%) cats. Two pathogens were detected in 15 (28.3%) cats, and three pathogens were detected in only 1 (1.9%)

Table 1. The details of real-time PCR for the detection of feline respiratory pathogens

Pathogen	Target gene	Real-time PCR conditions		
		PCR protocol	Primer/Probe concentration	LOD‡ (Based on Ct 40)
FCV	ORF1	RT-PCR*	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
Influenza A/B	M/NP	RT-PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
Feline reovirus	L3	RT-PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
FHV	gB	PCR†	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
P. multocida	toxA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
M. catarrhalis	copB	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
Bartonella spp.	ITS	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
C. felis	OmpA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
Aspergillus spp.	ITS1	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
M. felis	ssr	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
B. bronchiseptica	FhaB	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
Cryptococcus spp.	cyt b	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
P. carinii	DHFR	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
H. capsulatum	mtSSU	RT-PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
B. dermatitidis	BAD1	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx

*RT-PCR thermal condition: 50℃, 15 min～95℃, 5 min (95℃, 10s～60℃, 30s; 45 cycles).
†PCR thermal condition: 95℃, 5 min (95℃, 10s～60℃, 30s; 45 cycles).
‡LOD (limitation of detection) was determined to be 10 folds serial dilutions of synthetic plasmid including target gene of individual pathogen based on a CT value of 40.
FCV, Feline calicivirus; FHV, Feline herpesvirus; *P. multocida*, *Pasteurella multocida*; *M. catarrhalis*, *Moraxella catarrhalis*; *C. felis*, *Chlamydophila felis*; *M. felis*, *Mycoplasma felis*; *B. bronchiseptica*, *Bordetella bronchiseptica*; *P. carinii*, *Pneumocystis carinii*; *H. capsulatum*, *Histoplasma capsulatum*; *B. dermatitidi*, *Blastomyces dermatitidis*.

cat (Table 2).

Based on the living environment, 35 (68.6%) cats living outdoors and 18 (36.7%) cats living indoors were infected. Regarding the vaccination status of the 53 positive cats, 10 (18.9%) cats were vaccinated with Nobivac Fivecat (MSD, USA), 32 (60.4%) cats were not vaccinated, and 11 (20.8%) cats had unknown statuses. Among the 35 positive cats living outdoors, 4 (11.4%) were vaccinated, 26 (74.3%) were not vaccinated, and 5 (14.3%) had no related records (Table 3). Six pathogens were detected: *M. felis*, *P. multocida*, FHV, FCV, *B. bronchiseptica*, and *C. felis*. In 53 cats, including those with simple and multiple infections, *M. felis* was detected in 29 (54.7%) cats, followed by *P. multocida* in 18 (34.0%) cats, FHV in 10 (18.9%) cats, FCV in 7 (13.2%) cats, *B. bronchiseptica* in 3 (5.7%) cats, and *C. felis* in 2 (3.8%) cats. *M. felis* was the most common species in 29 (54.7%) cats, including 20 (57.1%) living outdoors and 9 (50.0%) living indoors.

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In addition, pathogens other than *C. felis* were detected at a much higher rate in cats living outdoors than in cats living indoors. These results were closely related to the detection rate of pathogens and the living environment (Table 4).

Clinical signs were observed in 37 cats that tested positive for a single pathogen, with nasal discharge and sneezing in 13 (35.1%), conjunctivitis in 6 (16.2%), drooling in 5 (13.5%), and dyspnea in 3 (8.1%), while 10 (27.0%) were asymptomatic. Based on the detected pathogens, 15 (40.5%) cats infected with *M. felis* and 10 (27.0%) cats infected with *P. multocida* displayed clinical signs shown in Table 4. Six pathogens commonly caused nasal discharge and sneezing. Despite being infected with a pathogen, 10 (27.0%) of the 37 cats were asymptomatic. Therefore, even if no URTD signs were observed, major pathogens related to the URTD could be detected (Table 5).

Of the 29 cats detected with *M. felis*, 22 (75.9%) showed signs of URTD, and 7 (24.1%) were without URTD signs. The number of sick cats increased with

Table 2. Type of pathogen detected in 53 of 100 cats using real-time PCR

Type	No. of cat positive (%)
Single pathogen	37 (69.8)
Two pathogens	15 (28.3)
Three pathogens	1 (1.9)
Total	53 (100.0)

Table 3. The number of cats infected with pathogens according to living environment and vaccination status in 53 positive cats

	Living environment		Total (*n*=100)
	Outdoor (*n*=51)	Indoor (*n*=49)	
Negative cats	16 (31.4%)	31 (63.3%)	47 (47.0%)
Positive cats	35 (68.6%)	18 (36.7%)	53 (53.0%)
Vaccine*			
Vaccinated	4 (11.4%)	6 (33.3%)	10 (18.9%)
Unvaccinated	26 (74.3%)	6 (33.3%)	32 (60.4%)
Unknown	5 (14.3%)	6 (33.3%)	11 (20.8%)

*Significant differences for vaccination status are found between outdoor and indoor group (P=0.237).

Table 4. Proportion of pathogens by outdoor and indoor in 53 positive cats

	Living environment		Total (*n*=100)
	Outdoor (*n*=51)	Indoor (*n*=49)	
Positive cats	35 (68.6%)	18 (36.7%)	53 (53.0%)
Pathogen*			
M. felis	20 (57.1%)	9 (50.0%)	29 (54.7%)
P. multocida	12 (34.3%)	6 (33.3%)	18 (34.0%)
FHV	7 (20.0%)	3 (16.7%)	10 (18.9%)
FCV	6 (17.1%)	1 (5.6%)	7 (13.2%)
B. bronchiseptica	2 (5.7%)	1 (5.6%)	3 (5.7%)
C. felis	0 (0.0%)	2 (11.1%)	2 (3.8%)
Total number of pathogens detected	47 (68.1%)	22 (31.9%)	69 (100.0%)

*Significant differences for pathogens are found between outdoor and indoor group (P=0.114).

Table 5. Major clinical sign and pathogen types in 37 cats infected with single pathogen

	Nasal discharge, sneezing (n=13)	Conjunctivitis (n=6)	Drooling (n=5)	Dyspnea (n=3)	Asymptomatic (n=10)	Total (n=37)
M. felis	5 (38.5%)	2 (33.3%)	2 (40.0%)	2 (66.7%)	4 (40.0%)	15 (40.5%)
P. multocida	3 (23.1%)	1 (16.7%)	2 (40.0%)	1 (33.3%)	3 (30.0%)	10 (27.0%)
FHV	2 (15.4%)	2 (33.3%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	5 (13.5%)
FCV	1 (7.7%)	0 (0.0%)	1 (20.0%)	0 (0.0%)	2 (20.0%)	4 (10.8%)
B. bronchiseptica	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.7%)
C. felis	1 (7.7%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.4%)

double infections. Triple infection with *M. felis*, FHV, and FCV was identified in only 1 (4.5%) cat, which displayed signs (Table 6).

Age-related pathogen detection rates were compared among the 53 positive cats. Of the 53 cats, 10 (18.9%) were under 1 year of age, 26 (49.1%) were 1~3 years of age, and 17 (32.1%) were over 3 years of age. *M. felis* was detected in all age groups, and both *P. multocida* and FHV were detected more in over 1 year of age than under 1 year of age. *B. bronchiseptica* was detected in 2 (20.0%) cats younger than 1 year and 1 (5.9%) cat over 3 years of age. *C. felis* was detected in 2 (7.7%) cats only at 1~3 years of age. There were no significant differences in pathogen detection among the age groups (Table 7).

DISCUSSION

This study investigated the pathogens related to URTD in Korean cats using real-time PCR. Various pathogens such as *M. felis*, *P. multocida*, FHV, FCV, *B. bronchiseptica*, and *C. felis* were analyzed. Similar to other studies, the detection rate of a single pathogen was high, but complex infections were identified (Cao et al, 2002; Berger et al, 2015; Litster et al, 2015; Maazi et al, 2016; Lobova et al, 2019; Walter et al, 2020). In addition, in this study, the PCR detection rate of each pathogen in cats with URTD was higher than without URTD (Helps et al, 2005; Low et al, 2007; Berger et al, 2015; Lobova et al, 2019). Accurate and fast tools, such as real-time PCR, can be helpful in URTD diagnosis.

According to the results of this study, *M. felis*, *P. multocida*, FHV, FCV, and *B. bronchiseptica* had approximately twice the prevalence rate in outdoor cats as in indoor cats. In other words, the detection rate of feline URTD pathogens is predicted to be closely related to the living environment. In cats that were positive for pathogens, the detection rate was three times higher in cats that were not vaccinated than in those that were vaccinated. Since vaccinated cats are also identified to be positive for pathogens, vaccination does not completely prevent pathogen infection; however, it is thought that the risk of infection can be greatly reduced.

Similar to other studies, *M. felis* was commonly detected as a cause of URTD and was the most common pathogen of complex infections. In addition, *M. felis* has been detected in similar numbers between age groups (Lobova et al, 2019). In addition, *M. felis* has been detected in 5-month-old Korean cats (Hong et al, 2015). It

Table 6. Distribution of single and multiple infections of *M. felis* in 29 cats with or without upper respiratory tract disease (URTD) signs*

Pathogens	With URTD signs (*n*=22)	Without URTD signs (*n*=7)
M. felis	11 (50.0%)	4 (57.1%)
M. felis+*P. multocida*	5 (22.7%)	2 (28.6%)
M. felis+FHV	2 (9.1%)	0 (0.0%)
M. felis+FCV	2 (9.1%)	0 (0.0%)
M. felis+*B. bronchiseptica*	1 (4.5%)	1 (14.3%)
M. felis+FHV + FCV	1 (4.5%)	0 (0.0%)

*Significant differences for pathogens are found between with URTD signs and without URTD signs group (*P*=0.088).

Table 7. Distribution by the age of 53 positive cats with pathogens*

Pathogens	Up to 1 year (*n*=10)	1 to 3 years (*n*=26)	Over 3 years (*n*=17)	Total (*n*=53)
M. felis	8 (80.0%)	12 (46.2%)	9 (52.9%)	29 (54.7%)
P. multocida	3 (30.0%)	7 (29.6%)	8 (47.1%)	18 (34.0%)
FHV	2 (20.0%)	7 (29.6%)	1 (5.9%)	10 (18.9%)
FCV	2 (20.0%)	3 (11.5%)	2 (11.8%)	7 (13.2%)
B. bronchiseptica	2 (20.0%)	0 (0.0%)	1 (5.9%)	3 (5.7%)
C. felis	0 (0.0%)	2 (7.7%)	0 (0.0%)	2 (3.8%)
Total number of pathogens detected	17 (24.6%)	31 (44.9%)	21 (30.4%)	69 (100.0%)

*Significant differences are found between <1 year and 1~3 years group (*P*=0.146).
*Significant differences are found between 1~3 years and ≥3 years group (*P*=0.251).
*Significant differences are found between <1 year and ≥3 years group (*P*=0.370).

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is considered a harmful pathogen for life from an early age. In this study, FHV, FCV, and *C. felis* were mainly detected at more than 1 year of age, and *B. bronchiseptica* was detected at less than 1 year of age, although the number of positive cats was small. In other studies, these pathogens were detected in cats younger than one year of age (Maazi et al, 2016; Lobova et al, 2019). Because infection with these pathogens can occur regardless of age, vaccination and immunization management can be an important means of preventing URTD.

Unlike the results reported in Korea in 2008 (Kang and Park, 2008), *M. felis* had the highest infection rate among Korean cats. In particular, the detection rate was the highest in individuals living outdoors, regardless of age, who exhibited URTD signs. In addition, a very high infection rate of *M. felis* was observed. In the case of *M. felis* complex infection, there were approximately three times more cats with clinical signs than cats without signs. In this study, triple infections (*M. felis*, FHV, and FCV) were observed in one cat that also showed URTD signs. In reaction to the high infection rate of *M. felis* in this study, further epidemiological studies on *M. felis* infection using a larger number of Korean cats are needed. Moreover, since URTD caused by *M. felis* infection has a long and complicated treatment process, having a prevention method will be helpful.

P. multocida, which can exacerbate stomatitis or conjunctivitis due to oral wounds or decreased immunity, had a high detection rate of 34% (18 cats) in this study. Additionally, 10 out of 18 cats were infected with only *P. multocida*, and seven and three cats showed signs of URTD or were asymptomatic, respectively. Two cats were diagnosed with stomatitis and pneumonia. Many individuals infected with *P. multocida* show clinical signs of URTD, especially in cats of various ages that live outdoors. Although *P. multocida* is normal bacterial flora, it has been identified as a cause of URTD signs in decreased immunity and poor living environment.

FHV is most frequently detected in URTD (Kang and Park, 2008; Litster et al, 2015; Schulz et al, 2015; Maazi et al, 2016; Walter et al, 2020). Although FHV can infect all cats, it has been reported that young cats and cats with chronic diseases show more severe signs (Sykes et al, 1999). Unlike the results of another study on Korean cats, the clinical signs observed in this study were nasal discharge, sneezing, and conjunctivitis. The detection rate of FHV was two times higher in cats living outdoors than indoors. Since FHV is highly contagious, vaccination is important to prevent URTD in homes and shelter environments.

Like other pathogens, FCV was detected more frequently in cats living outdoors under poor conditions than indoors. Clinical signs were observed when infected with FCV, and complex infections with *M. felis* and FHV also occurred, as in other studies (Berger et al, 2015; Lobova et al, 2019). Drooling due to an oral ulcer, which is a typical clinical sign of FCV, was observed. In this study, FCV occurred at all ages, similar to another study (Lobova et al, 2019). Although FCV infection does not appear to be closely related to age, the number of samples in this study was small; therefore, further studies are needed.

Some studies on *B. bronchiseptica* have reported that although it is common in kennels and shelter, infections are mostly asymptomatic or mild, and the chances of infection are much lower if living alone or in small groups (Litster et al, 2015; Lobova et al, 2019; Walter et al, 2020). Since the detection rate of *B. bronchiseptica* in this study was low at 6% (3 cats), more cases are needed to compare with the results of other studies.

C. felis is a zoonotic pathogen and its role in feline URTD has diminished. However, *C. felis* was the most common pathogen in feline conjunctivitis and URTD (Cao et al, 2002), and in another study *it* mainly infected kittens (1~3 months in age) (Maazi et al, 2016). In this study, it was detected in two cats (4%) living indoors, both at 1~3 years of age. The main clinical signs were sneezing and conjunctivitis. Unfortunately, the positivity rate of *C. felis* in this study was low, making it difficult to compare with that of other studies, However, the results of this study seem to differ from those of previous studies.

This is the first study conducted on Korean cats using several variables, including various ages, indoor cats with owners and outdoor cats (stray), and vaccination status, to detect pathogens that cause feline URTD. The real-time PCR test used in this study was useful for detecting feline URTD. Although the number of cats that participated in the study was small, we believe that the results of this study can provide valuable data on the prevalence of URTD in Korean cats.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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