KJVS
Korean Journal of Veterinary Service
https://doi.org/10.7853/kjvs.2022.45.2.101
pISSN: 1225-6552 eISSN: 2287-7630
Korean J Vet Serv 2022;45(2):101-110

ORIGINAL ARTICLE

Epidemiologic investigation of gastrointestinal pathogens for Korean cats with digestive sign

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This study was performed to investigate infectious gastrointestinal diseases in 115 Korean cats (83 indoors and 32 outdoors) with digestive signs such as diarrhea, anorexia or abdominal distention. Detection of infectious pathogens was analyzed using real-time PCR. As a result, 85 of 115 Korean cats were detected with feline corona virus (FCoV), feline parvo virus, Group A rotavirus, *Clostridium perfringens* (*C. perfringens*), *Campylobacter coli* (*C. coli*), *Campylobacter jejuni*, enterotoxigenic *Escherichia coli*, enteropathogenic *Escherichia coli*, *Salmonella* spp., *Tritrichomonas foetus*, *Cyclospora cayetanensis*, and *Giardia lamblia*. The most frequently detected pathogen was *C. perfringens* (52 cats, 61.2%), followed by FCoV (43 cats, 50.6%) and *C. coli* (16 cats, 18.8%). Also, single infection was the most common (43 cats), followed by double infection in 31 cats, triple infection in 7 cats, and quadruple infection in 4 cats. There was no significant relationship between pathogen detection and age, gender, living environment, weather, and diarrhea. However, there was a significant difference between the age group under 1 year and the age group 1~7 (P value<0.05). In this study, cats with suspected gastrointestinal infection were randomly evaluated, and other factors that could affect pathogen detection were insufficiently considered. For this reason, additional epidemiological investigations with a larger number of cats and sufficient consideration of the causes that may affect the results are needed. Nevertheless, it is thought that this study can also provide valuable information on gastrointestinal pathogens in Korean cats.

Key Words: Digestive system, Korean cat, Real-time PCR, Gastrointestinal pathogens, Epidemiologic investigation

Received April 6, 2022
Revised June 15, 2022
Accepted June 16, 2022

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INTRODUCTION

Various pathogens are directly or indirectly involved in the cause of digestive signs. In other words, many viral, bacterial, and parasitic infections in the gastrointestinal tract can cause disease in cats (Hackett and Lappin, 2003). Therefore, it is very important to diagnose as accurately and quickly as possible. Real-time polymerase chain reaction (real-time PCR) test of feces is frequently evaluated in cats with suspected gastrointestinal infection. Particularly, testing for infectious pathogens is necessary with or without diarrhea (Cho, 2017). In addition, both clinical and epidemiological relationships should be included in the evaluation of PCR test results. This is because it is often uncertain whether the disease is the result of a single pathogen or is due to coinfection with viral, bacterial, or parasitic pathogen (Paul and Stayt, 2019). Therefore, when reliable information about the pathogen is provided, it will help determine the appropriate treatment and the patient's prognosis.

Feline viral gastroenteritis is considered one of the most common diseases worldwide, especially in cats under the age of 1 who live in high-density environments such as cat shelters. Particularly, feline corona virus (FCoV) and feline parvo virus (FPV) are the most important viral causes of gastroenteritis, and Group A rotavi-

rus is also sporadically detected in the feces of cats with enteritis. Because FCoV infection can be asymptomatic and this condition can persist for up to 1 year or longer, seropositive rates reach up to 90% in multi-cat environment (Chang et al, 2010). However, according to other study, the prevalence of FCoV in Korea is 13.7% (29/212 cats), which is not very high compared to other countries (An et al, 2011). In Korea, FPV infection is one of the most important viral diseases in cats. It is mainly transmitted to infected cats through direct or excretory contact and is associated with high mortality and morbidity. According to other studies, morbidity and mortality are highest in cats younger than 1 year of age, and the severity of clinical signs varies with age, immune status, nutritional status, and coinfection status (Kim et al, 2013b; Di Martino et al, 2019). Group A rotavirus is known to infect mammals most extensively. However, in a large epidemiological study conducted in the UK, feline rotavirus did not show statistical significance with diarrhea or age (Di Martino et al, 2019).

Bacterial pathogens are frequently detected in feline feces. Among them, *Clostridium perfringens* (*C. perfringens*) is frequently detected in intestinal disease of many animals. *C. perfringens* type A is the most common genotype in cats and is suspected of being associated with mild diarrhea to severe hemorrhagic enteritis (Singer, 2010; Mark et al, 2011; Silva and Lobato, 2015). *Campylobacter coli* (*C. coli*) and *Campylobacter jejuni* (*C. jejuni*) are associated with intestinal disease in cats. Disease caused by *C. jejuni* have been found to be more likely to occur in young animals than in adults. In another study, *C. jejuni* was detected twice as high in animals with diarrhea as in those without diarrhea, but this association was not observed in animals older than 1 year of age (Marks et al, 2011). *Salmonella* spp. is an important pathogen of zoonotic disease and can be transmitted through contact with contaminated dry food, etc. In another study, the prevalence of Salmonella was higher in cats living outdoors, such as cat shelters or stray cats, than in indoor cats (Marks et al, 2011). *Escherichia coli* (*E. coli*) is part of the normal gut microbiome, but bacterial virulence factors and impaired local or systemic immunity can cause gastroenteritis. *E. coli* causing diarrhea has distinct pathogenic, clinical, pathological, and epidemiological characteristics. There are 7 types of pathogenicity including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), necrotoxigenic *E. coli* (NTEC), and diffusely adherent *E. coli* (DAEC). However, the role of these strains in cats is not well defined (Mark et al, 2011).

Tritrichomonas foetus (*T. foetus*) was recently reported to infect the gastrointestinal tract in cats (Hora et al, 2017). *T. foetus* lives in the ileum, cecum, and colon, and is known to cause chronic diarrhea through close contact with the intestinal epithelium. In another study, the intestinal prevalence of *T. fetus* in cats was 30.8% in the USA (36/117 cats), 14.4% in the UK (16/111 cats), 32.4% in Italy (24/74 cats), and 24.4% in Switzerland (11/45 cats). In Korea, the first clinical case of *T. fetus* infection was reported in 2008 (Lim et al, 2010). Giardia is a protozoan parasite that can infect the small intestine in cats and cause diarrhea. It is particularly common in young cats, and the main clinical signs are diarrhea and weight loss. (Tangtrongsup and Scorza, 2010; Gruffydd-Jones et al, 2013). *Cyclospora cayetanensis* (*C. cayetanensis*) is a parasite known to cause diarrhea and has many similarities to protozoan parasite such as *Cryptosporidium* spp.

Until recently, epidemiologic studies on infectious gastrointestinal pathogens in Korean cats using real-time PCR have not been sufficiently conducted. Therefore, this study was conducted to identify the distribution and prevalence of major infectious gastrointestinal pathogens in Korean cats with digestive signs.

MATERIALS AND METHODS

Target animal

This study was conducted to investigate infectious

https://doi.org/10.7853/kjvs.2022.45.2.101

gastrointestinal pathogens in 115 cats with 83 indoors (owned) and 32 outdoors (stray) with digestive signs such as diarrhea, anorexia, or abdominal distention. All cats were visited at Royal Animal Medical Center (Seoul, Korea) for 2 years from 2019 to 2020. Immediately after visiting the hospital, a general physical examination was performed after recording the breed, age, sex, living environment, and neutering of all cats. However, investigations on body weight, vaccination status, and underlying diseases were not recorded.

Fecal collection

For the detection of gastrointestinal pathogens from 115 cats, fresh feces were swabbed and immediately stored in UTM container at 4℃. The collected feces were transferred to the veterinary diagnostic laboratory (PobaniLab, Korea) within 24 hours and subjected to real-time PCR panel test.

Nucleic acid purification and real-time PCR

For real-time PCR, 150 μL of stool suspension was used for nucleic acid purification. Nucleic acids were extracted from samples using a total nucleic acid purification kit (POSTBIO, Guri, Korea) based on the QIA cube platform (Qiagen) using a protocol tailored after the user's extraction protocol with the best optimized conditions. For real-time PCR, 5 μL of nucleic acid was mixed with 20 μL of master mix from Qiagen for individual targets, and qPCR or qRT-PCR was performed using an Agilent AriaMx (Agilent, Santa Clara, CA, USA). All molecular assays were performed according to the standard laboratory instructions of PobaniLab.

Target gene and analytical sensitivity of qPCR or qRT-PCR

Target genes for gastrointestinal pathogen detection using real-time PCR included genes from FCoV (membrane protein) (Benetka et al, 2004), FPV (VP2) (Streck et al, 2013), feline immunodefciency virus (FIV) (gag) (Wilkes et al, 2015), Group A rotavirus (nsp4) (Liu et al, 2013), *C. perfringens* (α-toxin gene, cpa) (Ma et al, 2007), *C. coli* (gyrB), *C. jejuni* (rimM) (Antikainen et al, 2013), *Salmonella* spp. (invE) (Kim et al, 2013a), ETEC (ST and LT), EPEC (bfpA), EIEC) (ipaH), EHEC (stx1 and stx2) (Liu et al, 2013), *Giardia lamblia* (18s rRNA), *T. foetus* (ITS-1) (Yao, 2013), *Toxoplasma gondii* (RE) (Lin et al, 2000), *Cryptosporidium parvum* (18s rRNA), *Entamoeba histolytica* (18s rRNA) (Liu et al, 2013), *C. cayetanensis* (18s rRNA) (Varma et al, 2003), and *Toxocara cati* (ITS-1) (Durant et al, 2012). Because the pathogenicity of *E. coli* differs according to the subtype (Greene, 2013), we tested for ETEC, EPEC, EIEC, and EHEC (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). The analytical sensitivity (detection limit) is shown in Table 1.

Data analysis

Pathogen infection and coinfection rate of 115 cats were analyzed. In addition, it was analyzed whether there was a relationship between the detected pathogens according to age, sex, living environment, and the presence or absence of diarrhea. Ages were classified as less than 1 year old, 1 to 7 years old, and 7 years old or older. Sex was divided into male and female, including whether or not neutering. The living environment was classified as an indoor cat if living indoors with an owner, and an outdoor cat if not. In addition, it was classified according to the presence or absence of diarrhea.

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

Pathogen	Target gene	Real-time PCR conditions		
		PCR protocol	Primer/probe concentration	LOD[c] (based on Ct 40)
FCoV	M	RT-PCR[a]	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies
FPV	vp2	PCR[b]	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
FIV	gag	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
Group A rotavirus	nsp4	RT-PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
C. perfringens	cpa	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
C. coli	gyrB	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
C. jejuni	rimB	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
Salmonella spp.	invE	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
ETEC	ST/LT	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
EPEC	bfpA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
EIEC	ipaH	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
EHEC	stx1/stx2	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
G. lamblia	18s rRNA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	10 copies/Rx
T. foetus	ITS1	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
T. gondii	RE	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
C. parvum	18s rRNA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
E. histolytica	18s rRNA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
C. cayetanensis	18s rRNA	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx
T. cati	ITS1	PCR	Primer: 10 pmole/Rx Probe: 5 pmole/Rx	100 copies/Rx

FCoV, Feline corona virus; FPV, Feline parvo virus; FIV, Feline immunodeficiency virus; *C. perfringens*, *Clostridium perfringens*; *C. coli*, *Campylobacter coli*; *C. jejuni*, *Campylobacter jejuni*; ETEC, Enterotoxigenic *Escherichia coli*; EPEC, Enteropathogenic *Escherichia coli*; EIEC, Enteroinvasive *Escherichia coli*; EHEC, Enterohemorrhagic *Escherichia coli*; *G. lamblia*, *Giardia lamblia*; *T. foetus*, *Tritrichomonas foetus*; *T. gondii*, *Toxoplasma gondii*; *C. parvum*, *Cryptosporidium parvum*; *E. histolytica*, *Entamoeba histolytica*; *C. cayetanensis*, *Cyclospora cayetanensis*; *T. cati*, *Toxocara cati*.

[a]RT-PCR thermal condition: 50°C, 15 min～95°C, 5 min (95°C, 10 s～60°C, 30 s; 45 cycles).

[b]PCR thermal condition: 95°C, 5 min (95°C, 10 s～60°C, 30 s; 45 cycles).

[c]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.

https://doi.org/10.7853/kjvs.2022.45.2.101

RESULTS

The breeds of 115 cats were classified into 13, and Korean short hair cats accounted for the most with 70 (60.9%) cats, followed by Scottish Fold with 8 (7.0%) cats, and Russian Blue with 7 (6.1%) cats (Table 2). By age, 14 (12.2%) cats were less than 1 year old, 76 (66.1%) cats were 1~7 years old, and 25 (21.7%) cats were 7 years old or older. And, by sex. All 115 cats were neutered, and they were classified into 69 (60.0%) male and 46 (40.0%) female (Fig. 1). As a result of classification by living environment, there were 83 (72.2%) cats living indoors and 32 (27.8%) cats living outdoors. Diarrhea was observed in 59 (51.3%) cats.

In this study, gastrointestinal pathogens were detected in 85 (73.9%) of 115 cats, and the detected pathogens were 3 types of viruses (FCoV, FPV, Group A rotavirus), 6 types of bacteria (*C. perfringens*, *C. coli*, *C. jejuni*, ETEC, EPEC, *Salmonella* spp.), and 3 types of parasites (*T. foetus*, *C. cayetanensis*, *G. lamblia*). Among the 12 pathogens detected, *C. perfringens* infection was the most common in 52 (61.2%) cats, FCoV was detected in 43 (50.6%) cats, and *C. coli* was detected in 16 (18.8%) cats. Also, single infection in 43 (50.6%) of 85 cats, double infection in 31 (36.5%), triple infection in 7 (8.2%), and quadruple infection in 4 (4.8%) was confirmed. *C. perfringens* was most detected in single infection (51.2%, 22 cats) and triple infection (85.7%, 6 cats). FCoV was detected the most in double infection (77.4%, 24 cats) and quadruplicate infection (100.0%, 4 cats). Particularly, FCoV was detected in all 4 (100.0%) cats in quadruple infection. Cats infected with *C. perfringens* and FCoV were detected the most in single and double infection, respectively. FCoV, *C. perfringens*, *C. coli*, ETEC, and EPEC were detected in all types of infection (Table 3).

Table 2. Breed distribution in 115 Korean cats enrolled in the study

Breed	Cat no. (%)
Korean short hair	70 (60.9)
Scottish fold	8 (7.0)
Russian blue	7 (6.1)
Persian	5 (4.3)
American short hair	5 (4.3)
Bengal	4 (3.5)
British short hair	3 (2.6)
Ragdoll	3 (2.6)
Abyssinian	2 (1.7)
Siamese	2 (1.7)
Norwegian forest	2 (1.7)
American curi	2 (1.7)
Turkish angora	2 (1.7)

The distribution of detected pathogens by age and sex is as follows. When identifying the most detected pathogens by age, FCoV was detected in 5 (35.7%) of 14 cats under 1 year of age, *C. perfringens* in 37 (48.7%) of 76 cats 1 to 7 years of age, and *C. perfringens* in 12 (48.0%) of 25 cats over 7 years of age. There was a significant difference between the age group under 1 year and the age group 1~7 (P value<0.05, P=0.031). Pathogens detected at all ages were FCoV, FPV, *C. perfringens*, *C.*

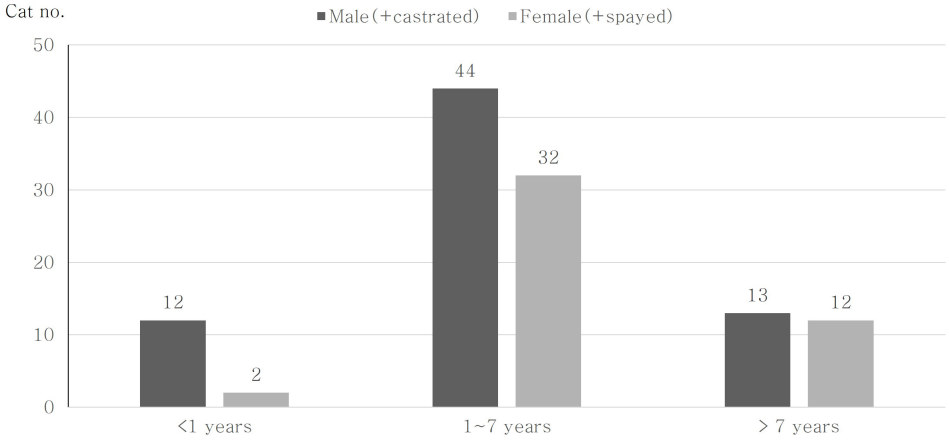

Fig. 1. Distribution of age and sex of 115 Korean cats involved in this study. By age, there were 14 cats under 1 year old, 76 cats between 1 to 7 years old, and 25 cats over 7 years old. And males were classified as 69 cats and females as 46 cats. All cats were neutered.

Table 3. Gastrointestinal pathogens and single or multiple infection types detected in 115 Korean cats using real-time PCR

Pathogen	Positive cats (n=85)	Infection type			
		Single (n=43)	Double (n=31)	Triple (n=7)	Quadruple (n=4)
FCoV	43 (50.6%)	11 (25.6%)	24 (77.4%)	4 (57.1%)	4 (100.0%)
FPV	5 (5.9%)	2 (4.7%)	2 (6.5%)	0 (0.0%)	1 (25.0%)
Group A rotavirus	2 (2.4%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (25.0%)
C. perfringens	52 (61.2%)	22 (51.2%)	21 (67.7%)	6 (85.7%)	3 (75.0%)
C. coli	16 (18.8%)	3 (7.0%)	7 (22.6%)	4 (57.1%)	2 (50.0%)
C. jejuni	4 (4.7%)	0 (0.0%)	2 (6.5%)	2 (28.6%)	0 (0.0%)
ETEC	7 (8.2%)	1 (2.3%)	3 (9.7%)	1 (14.3%)	2 (50.0%)
EPEC	4 (4.7%)	1 (2.3%)	1 (3.2%)	1 (14.3%)	1 (25.0%)
Salmonella spp.	1 (1.2%)	0 (0.0%)	1 (3.2%)	0 (0.0%)	0 (0.0%)
T. foetus	3 (3.5%)	2 (4.7%)	0 (0.0%)	1 (14.3%)	0 (0.0%)
C. cayetanensis	3 (3.5%)	0 (0.0%)	1 (3.2%)	1 (14.3%)	1 (25.0%)
G. lamblia	2 (2.4%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	1 (25.0%)

FCoV, Feline corona virus; FPV, Feline parvo virus; *C. perfringens*, *Clostridium perfringens*; *C. coli*, *Campylobacter coli*; *C. jejuni*, *Campylobacter jejuni*; ETEC, Enterotoxigenic *Escherichia coli*; EPEC, Enteropathogenic *Escherichia coli*; *T. foetus*, *Tritrichomonas foetus*; *C. cayetanensis*, *Cyclospora cayetanensis*; *G. lamblia*, *Giardia lamblia.*

Table 4. Distribution of gastrointestinal pathogens according to age and sex in 115 Korean cats

Pathogen	Age#			Sex*	
	<1 year (n=14)	1～7 years (n=76)	≥7 years (n=25)	Male (n=69)	Female (n=46)
FCoV	5 (35.7%)	30 (39.5%)	8 (32.0%)	19 (27.5%)	24 (52.2%)
FPV	2 (14.3%)	2 (2.6%)	1 (4.0%)	3 (4.3%)	2 (4.3%)
Group A rotavirus	0 (0.0%)	1 (1.3%)	1 (4.0%)	0 (0.0%)	2 (4.3%)
C. perfringens	3 (21.4%)	37 (48.7%)	12 (48.0%)	31 (44.9%)	21 (45.7%)
C. coli	2 (14.3%)	11 (14.5%)	3 (12.0%)	12 (17.4%)	4 (8.7%)
C. jejuni	0 (0.0%)	4 (5.3%)	0 (0.0%)	2 (2.9%)	2 (4.3%)
ETEC	1 (7.1%)	4 (5.3%)	2 (8.0%)	2 (2.9%)	5 (10.9%)
EPEC	1 (7.1%)	1 (1.3%)	2 (8.0%)	2 (2.9%)	2 (4.3%)
Salmonella spp.	0 (0.0%)	1 (1.3%)	0 (0.0%)	1 (1.4%)	0 (0.0%)
T. foetus	0 (0.0%)	2 (2.6%)	1 (4.0%)	1 (1.4%)	2 (4.3%)
C. cayetanensis	0 (0.0%)	3 (3.9%)	0 (0.0%)	1 (1.4%)	2 (4.3%)
G. lamblia	0 (0.0%)	2 (2.6%)	0 (0.0%)	0 (0.0%)	2 (4.3%)

FCoV, Feline corona virus; FPV, Feline parvo virus; *C. perfringens*, *Clostridium perfringens*; *C. coli*, *Campylobacter coli*; *C. jejuni*, *Campylobacter jejuni*; ETEC, Enterotoxigenic *Escherichia coli*; EPEC, Enteropathogenic *Escherichia coli*; *T. foetus*, *Tritrichomonas foetus*; *C. cayetanensis*, *Cyclospora cayetanensis*; *G. lamblia*, *Giardia lamblia*.
#Significant differences are found between <1 year and 1～7 years group (*P* value=0.031).
#Significant differences are found between 1～7 years and ≥7 years group (*P* value=0.0695).
#Significant differences are found between <1 year and ≥7 years group (*P* value=0.1333).
*Significant differences are found between male and female group (*P* value=0.446).

coli, ETEC, and EPEC. When the most detected pathogens were identified by sex, *C. perfringens* was detected in 31 (44.9%) of 69 cats in males and FCoV in 24 (52.2%) of 46 cats in females. Except for Group A rotavirus, *Salmonella* spp., and *G. lamblia*, other pathogens were detected in both males and females (Table 4).

The distribution of detected pathogens by living environment and weather is as follows. When the pathogens most detected were identified by living environment, *C. perfringens* was found in 36 (43.4%) of 83 indoor cats and in 16 (50.0%) of 32 outdoor cats, respectively. There was no significant relationship between indoor and

https://doi.org/10.7853/kjvs.2022.45.2.101

Table 5. Distribution of gastrointestinal pathogens according to living environment and weather in 115 Korean cats

Pathogen	Living environment#		Weather*	
	Indoor (n=83)	Outdoor (n=32)	Warm (n=59)	Cold (n=56)
FCoV	29 (34.9%)	14 (43.8%)	25 (42.4%)	18 (32.1%)
FPV	2 (2.4%)	3 (9.4%)	4 (6.8%)	1 (1.8%)
Group A rotavirus	2 (2.4%)	0 (0.0%)	1 (1.7%)	1 (1.8%)
C. perfringens	36 (43.4%)	16(50.0%)	26 (44.1%)	26 (46.4%)
C. coli	10 (12.0%)	6 (18.8%)	6 (10.2%)	10 (17.9%)
C. jejuni	2 (2.4%)	2 (6.3%)	1 (1.7%)	3 (5.4%)
ETEC	5 (6.0%)	2 (6.3%)	3 (5.1%)	4 (7.1%)
EPEC	3 (3.6%)	1 (3.1%)	2 (3.4%)	2 (3.6%)
Salmonella spp.	0 (0.0%)	1 (3.1%)	1 (1.7%)	0 (0.0%)
T. foetus	3 (3.6%)	0 (0.0%)	1 (1.7%)	2 (3.6%)
C. cayetanensis	1 (1.2%)	2 (6.3%)	3 (5.1%)	0 (0.0%)
G. lamblia	1 (1.2%)	1 (3.1%)	1 (1.7%)	1 (1.8%)

FCoV, Feline corona virus; FPV, Feline parvo virus; *C. perfringens*, *Clostridium perfringens*; *C. coli*, *Campylobacter coli*; *C. jejuni*, *Campylobacter jejuni*; ETEC, Enterotoxigenic *Escherichia coli*; EPEC, Enteropathogenic *Escherichia coli*; *T. foetus*, *Tritrichomonas foetus*; *C. cayetanensis*, *Cyclospora cayetanensis*; *G. lamblia*, *Giardia lamblia*.
#Significant differences are found between indoor and outdoor group (*P* value=0.16).
*Significant differences are found between warm and cold group (*P* value=0.445).

outdoor group (*P* value<0.05, *P*=0.16). When the most detected pathogens were identified by weather, *C. perfringens* was detected in 26 (44.1%) of 59 cats in warm weather and in 26 (46.4%) of 56 cats in cold weather, respectively. There was no significant relationship between warm and cold group (*P* value<0.05, *P*=0.445). In the distribution by living environment and weather, *C. perfringens* was detected the most (Table 5).

In clinical evaluation, diarrhea was observed in only 59 (51.3%) of 115 cats. Twelve pathogens detected were also known to cause gastrointestinal signs, but not necessarily diarrhea in all cats. In addition, no significance was observed between the detected pathogen and the presence or absence of diarrhea (*P* value<0.05, *P*=0.426) (Table 6).

Table 6. Detection rates of gastrointestinal pathogens in 115 Korean cats with or without diarrhea

Pathogen	Diarrhea (n=59)#	No diarrhea (n=56)#
FCoV	23 (39.0%)	20 (35.7%)
FPV	2 (3.4%)	3 (5.4%)
Group A rotavirus	0 (0.0%)	2 (3.6%)
C. perfringens	26 (44.1%)	26 (46.4%)
C. coli	5 (8.5%)	11 (19.6%)
C. jejuni	2 (3.4%)	2 (3.6%)
ETEC	2 (3.4%)	5 (8.9%)
EPEC	3 (5.1%)	1 (1.8%)
Salmonella spp.	1 (1.7%)	0 (0.0%)
T. foetus	1 (1.7%)	2 (3.6%)
C. cayetanensis	2 (3.4%)	1 (1.8%)
G. lamblia	0 (0.0%)	2 (3.6%)

FCoV, Feline corona virus; FPV, Feline parvo virus; *C. perfringens*, *Clostridium perfringens*; *C. coli*, *Campylobacter coli*; *C. jejuni*, *Campylobacter jejuni*; ETEC, Enterotoxigenic *Escherichia coli*; EPEC, Enteropathogenic *Escherichia coli*; *T. foetus*, *Tritrichomonas foetus*; *C. cayetanensis*, *Cyclospora cayetanensis*; *G. lamblia*, *Giardia lamblia*.
#Significant differences are found between diarrhea and no diarrhea group (*P* value=0.426).

DISCUSSION

In this study, the types and detection rates of gastrointestinal pathogens detected in 115 Korean cats suspected of infectious gastrointestinal disease were observed.

FCoV is one of the most common infectious agents in the feline gastrointestinal tract, and in other study, the prevalence of FCoV in Korean cats was not high, and researchers predicted that the prevalence of FCoV

would increase as the number of cats increases in the future (An et al, 2011). As expected, the results of this study identified FCoV as the second most common infection, and unusually, it was identified as the largest cause of double infection. In addition, FCoV was detected even without diarrhea, and since FCoV can be infected either acutely or chronically, even after gastrointestinal signs disappear, FCoV is often continuously excreted in feces. So, the cat recovering from FCoV also is required real-time PCR test for feces (Paul and Stayt, 2019).

FPV is known to be a highly contagious, high-mortality virus in cats and causes enteritis in the digestive tract. Similar to the results of other study, the detection rate of FPV was low (5 of 85 cats, 5.9%) (Kim et al, 2013b), but the detection rate was about 4 times higher in cats less than 1 year old or living outdoors than in other groups. Group A rotavirus is considered of low importance in infectious diseases in small animal clinics, and 2 (2.4%) of 85 cats were detected in this study as well, and the infection is likely to resolve spontaneously.

Other study has also identified *C. perfringens* as the most common gastrointestinal pathogen. Similar rates were reported in cats with and without diarrhea (Mark et al, 2011). In this study, *C. perfringens* was found to be detected the most, and the diarrhea rate was similar to the results of other study.

Few studies have compared the prevalence of *Campylobacter* spp. in cats. In this study, diarrhea was observed in 7 (11.9%) of 85 cats. The infection rate (18.8%) of *C. coli* was the same as in other study (Marks et al, 2011). Although *C. jejuni* pathogen was found to be more likely to infect in young cats, but this study differed in that the pathogen was detected only in 1 to 7 years of age.

ETEC and EPEC are *E. coli* that can cause diarrhea and are usually undetectable. In this study, 2 (3.4%) of 85 cats with ETEC and 3 (5.1%) of 85 cats with EPEC had diarrhea. The rates of diarrhea due to ETEC and EPEC infection were similar to those of previous study (Oh et al, 2021).

Other study has shown a high prevalence of *Salmonella* spp.. The infection rate is higher in outdoor cats, such as shelters or stray cats, than indoor cats. In this study, since *Salmonella* spp. was only detected in 1 cat, it is difficult to compare with other study, but this study was detected in outdoor cats.

Although studies on feline gastrointestinal parasites are in process, there is currently insufficient information on *T. foetus* and *C. cayetanensis* in Korea (Lim et al, 2010). In this study, *T. foetus* and *C. cayetanensis* were detected in 3 (3.5%) of 85 cats each. If the study is conducted on more Korean cats, more meaningful results are expected. In a previous study, *Giardia* spp. had a higher prevalence in cats under 7 years of age, and similar result was found in this study (Oh et al, 2021).

C. coli, *C. jejuni*, *Salmonella* spp., and *G. lamblia* identified in this study are well-documented zoonoses that can cause disease in people and cats with low immunity. Although no clinical signs have been observed in cats in which these pathogens have been detected, caution is warranted as infection may occur directly or through excretory contact with cats.

The reasons why the results of this study are different from those published in 2017 in Korea are as follows. In this study, indoor cats were 2.6 times more likely than outdoor cats. Factors that could affect the detection of pathogens, such as whether vaccination was performed or changes in pathogens caused by previously administered drugs, were not sufficiently considered. Also, there may be differences in the detection rate due to the small number of cats in this study (Cho, 2017). In conclusion, since this study was a randomized evaluation of Korean cats suspected of infectious gastrointestinal disease, consideration of other causes was insufficient. However, such epidemiologic studies in Korean cats have not been conducted so far, so the results on the pathogen and detection rate of infectious gastrointestinal diseases obtained in this study are evaluated as very meaningful. Also, if more results are added in the future, the results of epidemiological investigation are expected to be more valuable. In addition, it is expected that the re-

https://doi.org/10.7853/kjvs.2022.45.2.101

sults of comparing the pathogen detection rate through a method other than real-time PCR with the results of this study will be meaningful.

ACKNOWLEDGEMENTS

This research was funded by Project No. PJ01690702 from the Rural Development Administration, Republic of Korea.

CONFLICT OF INTEREST

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

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https://doi.org/10.7853/kjvs.2022.45.2.101