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SHORT COMMUNICATION

Molecular screening of Feline bocaviruses (FBoVs) from captured wild felids in Korea

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Feline bocavirus (FBoV) is considered an emerging pathogen recently identified in domestic cats worldwide. To date, three species of FBoVs (FBoV-1, FBoV-2, and FBoV-3) have been reported, but there are no reports identifying FBoVs in Korea. In this study, we detected novel FBoVs for the first time in Korea in captive wild felids (four European lynx and a lion) kept at Seoul Zoo. In FBoV-positive fecal samples, not only singular infections but also dual or triple infections with three different species of FBoVs were confirmed, suggesting that three species of FBoVs are already introduced and co-circulated in susceptible host animals in Korea. These results will help expand our understanding of the geographical distribution and host susceptibility of novel FBoVs. Further studies are necessary to determine the infection status of FBoVs in domestic cats and the genetic characteristics of the viruses circulating in Korea.

Key Words: Feline bocavirus, Molecular screening, European lynx, Lion, Korea

INTRODUCTION

Parvoviruses in the family *Parvoviridae* are currently classified into three subfamilies, *Densovirinae*, *Parvovirinae*, and *Hamaparvovirinae*, members of which infect non-vertebrate, vertebrate, and both non-vertebrate and vertebrate hosts, respectively. The subfamily *Parvovirinae* is divided into eleven genera based on the recently revised taxonomy: *Amdoparvovirus*, *Artiparvovirus*, *Aveparvovirus*, *Bocaparvovirus*, *Copiparvovirus*, *Dependoparvovirus*, *Erythroparvovirus*, *Loriparvovirus*, *Protoparvovirus*, *Sandeparvovirus*, and *Tetraparvovirus* (Cotmore et al, 2019; Pénzes et al, 2020). Bocavirus (BoV), belonging to the genus *Bocaparvovirus* within the subfamily *Parvovirinae*, is considered an emerging pathogen and has been detected in a wide range of hosts, including humans, cats, cows, pigs, gorillas, chimpanzees, California sea lion, rabbits, Himalayan marmots, camels, wild boars, bats, minks, house shrews, Amur leopard cat, Eastern roe deer, and rodents (Aryal and Liu, 2021; Liu et al, 2022; Sun et al, 2023). Currently, members of BoV are classified into thirty-one species based on the ICTV classification criteria: *Bocaparvovirus carnivoran1* to *7*, *Bocaparvovirus chiropteran1* to 5, *Bocaparvovirus incertum1*, *Bocaparvovirus largomorph1*, *Bocaparvovirus pinniped1* and 2, *Bocaparvovirus primate1* to 3, *Bocaparvovirus rodent1* to 3, and *Bocaparvovirus ungulate1* to 9. Among these, *Bocaparvovirus carnivoran3*, *4*, and *5*, also known as feline bocavirus 1 (FBoV-1), FBoV-2, and FBoV-3, have been founded in domestic cats with variable clinical signs (Capozza et al, 2021; ICTV, 2024).

FBoV was first identified from stray cats in Hong Kong in 2012 (Lau et al, 2012), and then designated as a species named *Bocaparvovirus carnivoran3*, according to the new strict classification criteria of ICTV (Cotmore et

al, 2019; Pénzes et al, 2020). FBoV-2 was subsequently discovered in the feces of a single healthy cat from Portugal (Ng et al, 2014). The detected FBoV-2 POR1 strain was genetically distinct from FBoV-1 strains previously identified in Hong Kong and now classified as a different species, *Bocaparvovirus carnivoran4*. FBoV-3 was detected in fecal samples collected from sheltered cats in the United States in 2014 and was classified as the third FBoV (species *Bocaparvovirus carnivoran5*) because it showed low amino acid homology to previously identified FBoV-1 and FBoV-2 (Zhang et al, 2014). Since then, these three species of FBoVs have been identified in cats with and without clinical signs in several countries, including Europe, China, Japan, Thailand, Canada, and Australia, suggesting that genetically and biologically different FBoVs are spreading in cat populations around the world (Ng et al, 2014; Takano et al, 2016; Liu et al, 2018; Yi et al, 2018; Piewbang et al, 2019; Li et al, 2020; Van Brussel et al, 2022).

To date, BoV infections have been reported in humans (Chung et al, 2006), pigs (Choi et al, 2014; Yoo et al, 2015), and dogs (Choi et al, 2015; Koh et al, 2020) in Korea, but have not been reported in domestic cats and wild felids. In this study, molecular screening was carried out with fecal samples collected from five species of captured wild felids to identify novel FBoVs in Korea. These results will help expand our knowledge of epidemiology and virology of FBoVs in Korea.

MATERIALS AND METHODS

Clinical samples

A total of 35 fecal samples were collected from five different species of wild felids, including four Eurasian lynxes (*Lynx lynx*), twelve leopard cats (*Prionailurus bengalensis*), eleven Siberian tigers (*Panthera tigris*), three leopards (*Panthera pardus*), and five lions (*Panthera reo*) in the Seoul Zoo in 2024. All animals were clinically healthy without any clinical signs suspected with enteric diseases at the time of sample collection. The collected samples were stored at −80℃ until processing. The samples to be used in this study were collected by veterinarians at the Seoul Zoo without any animal contact, ethical approval from our institute regarding the animals was not required.

Nucleic acid extraction

Each collected fecal sample was immersed in 1 mL of phosphate-buffered saline (0.15 M, pH 7.2) and then centrifuged at 3000 × g for 10 min. Then, supernatants were aliquoted and stored at −80℃ for further analysis. Total nucleic acids were extracted from the 200 uL of collected swab samples using a TANBead Nucleic Acid Extraction Kit with a fully automated magnetic bead operating platform (Taiwan Advanced Nanotech Inc., Taoyuan, Taiwan), according to the manufacturer's directions. The extracted nucleic acids were allocated and stored at −80℃ until further use.

Molecular screening of feline bocaparvoviruses

Molecular screening for three species of FBoVs (FBoV-1, FBoV-2, and FBoV-3) was carried out with SYBR Green-based real-time quantitative polymerase chain reaction (qPCR) assays using each virus-specific primer and a commercial qPCR kit [RealHelix™ qPCR kit (Green), NanoHelix, Daejeon, Republic of Korea] according to the manufacturer's instructions. Previously described primers for FBoV-1 (Lau et al, 2012), FBoV-2 (Ng et al, 2014), and FBoV-3 (Zhang et al, 2014) were used in the qPCR assays (Table 1). The qPCR program was identical to the following conditions: initial denaturation at 95℃ for 15 min, followed by 40 cycles at 95℃ for 15 s, and 60℃ for 60 s for amplification. Fluorescence signals generated by SYBR Green dye for tested samples were measured at the end of each annealing step and the cycle threshold (Ct) values for each sample were calculated by determining the point at which the fluorescence exceeded the threshold limit. To interpret the qPCR results, samples with a Ct value of less than 37

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Table 1. Primers used in real-time PCR assay for screening of feline bocaparvoviruses (FBoV) in this study

Pathogen	Primer	Sequence (5'–3')	Amplicon size (bp)	Reference
FBoV-1	FBoV1F	TCTACAAGTGGGACATTGGA	133	Lau et al (2012)
	FBoV1R	GAGCTTGATTGCATTCACGA		
FBoV-2	FBoV2AF	TCGTTCGTCTTGGAACATAGC	331	Ng et al (2014)
	FBoV2AR	CAGAGCGTGGATCTGTCTGA		
FBoV-3	FBD1L2	CAAAGGATCGGGAGCGGGCG	388	Zhang et al (2014)
	FBD1R2	TGCCCATGGTGTTGTGATTCCTATCCA		

Table 2. Molecular detection of feline bocaparvoviruses (FBoV) in fecal samples of captive wild felids

Species	Common name	No. of samples	Detection of FBoV (No. of positive)		
			FBoV-1	FBoV-2	FBoV-3
Lynx lynx	Eurasian lynx	4	4	1	2
Prionailurus bengalensis	Leopard cat	12	0	0	0
Panthera tigris	Siberian tiger	11	0	0	0
Panthera pardus	Leopard	3	0	0	0
Panthera reo	Lion	5	0	1	1
Total		35	4	2	3

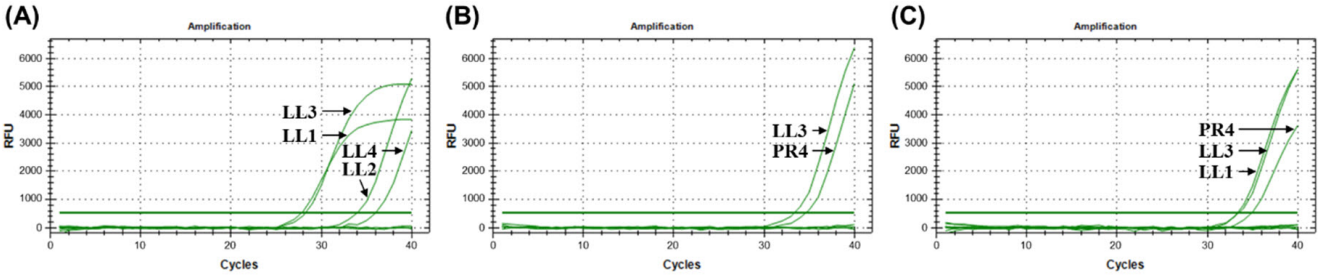

Fig. 1. Amplification curve of real-time PCR assay for feline bocaparvoviruses from wild felids. The positive signals of real-time PCR assay for feline bocaparvovirus 1 (FBoV-1) (A), FBoV-2 (B), and FBoV-3 (C) were generated with five fecal samples obtained from four European lynxes (*Lynx lynx*) and a lion (*Panthera reo*). Detailed information of positive samples is provided in Table 3.

were considered positive, whereas those with Ct values of higher than 37 were considered negative.

RESULTS AND DISCUSSION

In this study, we conducted molecular screening of feline infectious viral pathogens as part of disease monitoring on wild felids raised in the Seoul Zoo. Through this, we were fortunate to confirm that European lynx and lion in the zoo were exposed to novel FBoVs. All three species of FBoV DNAs were detected from five fecal samples collected from four European lynxes and a lion (Table 2). FBoV-1 DNAs were detected from all four fecal samples of European lynxes, FBoV-2 DNAs were detected from two fecal samples of a European lynx and a lion, and FBoV-3 DNAs were detected from three fecal samples from two European lynx and a lion, respectively (Table 2, Fig. 1). Furthermore, co-infection of FBoV-1, FBoV-2, and/or FBoV-3 also was identified in the FBoV-positive samples in this study (Table 3). These results showed that three species of FBoVs have already been introduced in Korea and are infected in some captive wild felids such as European lynx and lion as determined in this study.

As an apex predator, the Eurasian lynx (Lynx lynx) and lion (*Panthera reo*) play important and unique roles

Table 3. Details of diagnostic results for feline bocaparvovirus (FBoV)-positive samples

Species	Sample ID	Results of real-time PCR assay (Ct value)		
		FBoV-1	FBoV-2	FBoV-3
Lynx lynx (European lynx)	LL1	27.81	-	33.33
	LL2	34.00	-	-
	LL3	28.23	34.62	34.93
	LL4	36.06	-	-
Panthera reo (Lion)	PR4	-	33.36	33.46

-, negative results (>37 of Ct value)

in the natural functioning of ecosystems. These two species have been listed as "Least Concern" or "Critically Endangered" species on the International Union for the Conservation of Nature (IUCN) Red List, respectively (Ripple et al, 2014). Despite the efforts for species conservation, the life of the endangered feline species is threatened by some feline infectious pathogens worldwide. Previous studies showed that wild lynxes and lions are susceptible to several feline viral pathogens such as feline parvovirus, feline herpesvirus, feline calicivirus, feline leukemia virus, feline immunodeficiency virus, and canine distemper virus (Schmidt-Posthaus et al, 2002; Ramsauer et al, 2007; Meli et al, 2009; Roelke et al, 2009; Wasieri et al, 2009; Lane et al, 2016; Weckworth et al, 2020; Nájera et al, 2021; Ryser-Degiorgis et al, 2021). However, there have been few studies on the infectious viral disease in captive European lynx and lions in Korea. The finding in this study that FBoV DNAs were identified in fecal samples of European lynx and lion helps to expand our knowledge about the host range of the novel FBoVs and its epidemiology in wild felids.

The origin and transmission route of FBoVs detected in the lynxes and lion are unknown in this study. However, considering that interspecies transmission of feline pathogens between domestic cats and captive wild felids has been frequently reported in several countries, including Korea (Meli et al, 2009; Weckworth et al, 2020; Sacristán et al, 2021; Yeo et al, 2023), the FBoVs detected in wild felids in this study is presumed to have been transmitted from infected domestic cats. In this regard, it is worth noting a previous report that Siberian tigers at this zoo were infected with FPV due to cross-species transmission between tigers and stray cats roaming the zoo (Yeo et al, 2023). However, there are no studies on FBoV infections in the Korean domestic cat population yet. Therefore, further studies are urgently needed to investigate the infection status of FBoVs in Korean domestic cats.

Despite the wide distribution of the three species of FBoVs, co-infection cases of FBoV-1, FBoV-2, and/or FBoV-3 have been rarely reported in domestic cats (Ng et al, 2014; Takano et al, 2016; Liu et al, 2018; Piewbang et al, 2019; Li et al, 2020; Van Brussel et al, 2022). In a recent study carried out in China, co-infection cases of FBoV-1 and FBoV-2 were reported in domestic cats, although the co-infection rate of FBoV-1 and FBoV-2 was much lower than that of singular infection of FBoV-1 or FBoV-2 (Yi et al, 2018). Therefore, the co-infection status of three FBoVs was investigated by using each species-specific monoplex qPCR assays in this study. Of the five FBoV-positive samples, two were singularly infected with FBoV-1 (sample ID LL2 and LL4), but two were dually infected with FBoV-1 and FBoV-3 (sample ID LL1) or FBoV-2 and FBoV-3 (sample ID PR4). Surprisingly, one fecal sample collected from a Eurasian lynx (sample ID LL3) was triply infected with all three species of FBoVs (Table 3, Fig. 1). These results suggested that co-infections of two of three different species of FBoVs frequently occur in susceptible host populations. In this regard, more advanced multiplex qPCR assays are required for simultaneous and differential detection of three species of FBoVs from clinical samples in a single reaction.

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There are some limitations in this study. First, viral sequence analysis is important to characterize the Korean FBoVs detected in this study. However, our efforts for genetic sequencing of the detected FBoVs failed in this study due to the low viral loads (high Ct values) in the FBoV-positive clinical samples as shown in Table 3 and Fig. 1. Therefore, further studies are needed to elucidate the genetic characteristics of Korean FBoVs and their relationship with other FBoV strains reported in foreign countries. Second, the molecular screening of FBoVs in this study confirmed that the viruses are infected in susceptible wild felids in a Korean zoo, suggesting that the viruses might be circulating in the Korean domestic cat population. However, surveillance of the viruses in Korean domestic cats was not included in the scope of this study. Therefore, further epidemiological studies are required to investigate the infection status of FBoVs in the Korean domestic cat population. Furthermore, considering that various viral and bacterial pathogens are commonly co-infected in the cat population in Korea (Koh et al, 2020; Kim et al, 2022; Baek et al, 2023a; Baek et al, 2023b), further epidemiological studies are necessary to determine the co-infection status of the novel FBoV and other feline pathogens in Korean cat population.

In conclusion, we confirmed that three species of FBoVs were infected in two species of wild felids (*Lynx lynx* and *Panthera reo*) in this study. To the best of our author's knowledge, this is the first report on the detection of noble FBoVs from captive wild felids in Korea. These results will contribute to expanding our understanding of the geographical distribution and host susceptibility of the novel FBoVs. Further studies are necessary to investigate the infection status of FBoVs in domestic cats and the genetic characteristics of the viruses circulating in Korea.

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. This study was conducted in 2024 and was beyond the purview of the Institutional Animal Care and Use Committee (IACUC) at Kyungpook National University (KNU), as the KNU IACUC only evaluates proposals using laboratory animals maintained in indoor facilities and not research involving outdoor animals. The fecal samples from captive wild animals were collected by veterinarians working at Seoul Zoo's veterinary clinic without any restraint on the wild animals.

CONFLICT OF INTEREST

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

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