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Molecular characteristics of Budgerigar fledgling disease polyomavirus detected from parrots in South Korea

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ABSTRACT

Background: Budgerigar fledgling disease polyomavirus (BFDV) is the pathogen that causes budgerigar fledgling disease in psittacine species. The clinical signs of PBFV infection include ascites, hepatitis, and crop stasis. BFDV is associated with a high mortality rate in nestling birds. In contrast, adult birds only have mild symptoms such as feather dystrophy.
Objectives: This study aimed to determine the prevalence, genetic characteristics, and phylogenetic analysis of BFDV in pet parrots in Korea.
Methods: Fecal and tissue samples were collected from 217 pet parrots from 10 veterinary hospitals including Chungbuk National University Veterinary Hospital. The molecular screening was performed using polymerase chain reaction (PCR) analysis of the small t/large T antigen gene segment. Full-length genome sequencing with the Sanger and phylogenetic analysis were performed on BFDV-positive samples.
Results: The PCR results based on the small t/large T antigen gene marker indicated that BFDV DNA was present in 10 out of 217 screened samples. A whole-genome sequence was obtained from six strains and phylogenetic analysis revealed no significant relationship existed between the species and geographical locations amongst them.
Conclusions: The prevalence of BFDV infection in South Korea is not high when compared to the prevalence of BFDV in other parts of the world, however, it has been reported sporadically in various species and geographic locations. The whole-genome analysis revealed 0.2%–0.3% variation in intragenomic homogeneity among the six strains analyzed. Korean strains are separately on the phylogenetic tree from their counterparts from China and Japan which might reflect the substantial genetic variation.

Keywords: Budgerigar fledgling disease virus; molecular characterization; parrot; phylogenetic analysis; prevalence

INTRODUCTION

Budgerigar fledgling disease polyomavirus (BFDV), also known as avian polyomavirus, is a non-enveloped icosahedral DNA virus with a diameter of 40–45 nm. The genome is composed of double-stranded circular DNA with a size of approximately 5,000 nucleotides.

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Conflict of Interest
The authors declare no conflicts of interest.

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This virus belongs to species *Aves polyomavirus* 1, in the family *Polyomaviridae*. Within this polyomavirus group, Genus *Gammapolyomavirus* is the only avian virus genus. [1,2]. The genome of BFDV has divided into two parts: an early region and a late region. The early region encodes two non-structural proteins, large T antigen and small t antigen. On the other hand, the late region encodes structural proteins, VP1, VP2, VP3, and VP4 [3,4]. The genome also contains a non-coding control region which was located between the early and late regions. A non-coding control region contains promoter, polyadenylation signal, DNA replication origin, and enhancer regions [4,5]. Unlike mammalian polyomaviruses, which have well-defined tissue tropism and high host-specificity, avian polyomaviruses, such as BFDV, have low host specificity and can replicate in various organs; where they can be detected in all organs if infected [1,6]. Transmission of BFDV occurs both vertically and horizontally [7,8].

BFDV is the etiologic agent of budgerigar fledgling disease, an important immunosuppressive disease in different psittacine species [4,9,10]. However, this virus has also been reported in non-psittacine species, such as buzzards, falcons, and pigeons [11,12]. BFDV infection in nestlings is characterized by severe clinical signs including hepatitis, ascites, and hydropericardium as well as retardation of feather growth, abdomen swelling, crop stasis, and skin discoloration, resulting in the high mortality rate of up to 100%. However, BFDV infection in adults is only associated with mild symptoms such as feather dystrophies (French molt) [9,13]. BFDV infection has been reported in several species of psittacine birds on almost every continent, including countries such as China [4], Japan [14], Taiwan [2,15], Turkey [16], Pakistan [17], Thailand [18], Australia [19], New Zealand [20], Egypt [21], Germany [22], Czech Republic [23], Italy [24], Poland [25], Chile [26], Costa Rica [27], and USA [28]. In Korea, the virus was first detected in an Alexandrine parakeet (*Psittacula eupatria*) in 2014 [29], and in an African gray parrot (*Psittacus erithacus*) that was co-infected with Psittacine beak and feather disease, as well as and *Aspergillus fumigatus* and BFDV in 2017 [30]. However, to date, no information on the prevalence and genetic identity of the virus is available. This study aimed to determine the occurrence and genetic profile of BFDV in psittacine in Korea.

MATERIALS AND METHODS

Sample collection

From 2016 to 2019, a total of 217 samples were collected from five local veterinary hospitals in Seoul (18 samples), one in Gyeonggi (one sample), three in Busan (23 samples), and Chungbuk National university veterinary hospital (163 samples). The samples were submitted for the purpose of screening/detection of BFDV by molecular analysis. Of the total 217 samples, 199 samples were obtained from alive parrot droppings (135 samples from clinically healthy parrots, 42 samples from feather problems, 13 samples from gastrointestinal problems, four samples from non-specific problems, two samples from beak problems, two samples from neurological problems, and one sample from respiratory problem) and 18 from dead parrot tissues. Of the total 217 samples, 154 were from companion parrots, 58 were from pet shops, and five were from zoos. Samples were stored frozen at −20°C until processing for molecular analysis.

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DNA extraction and BFDV detection

DNA was extracted from tissues and fecal samples using a MagPurix Pathogen/Viral NAs B kit and MagPurix 12s automated nucleic acid purification system (Zinexts Life Science Corp., Taiwan) according to the manufacturer's instructions. DNA preparations were stored at −20°C until use. DNA preparations were screened for the presence of BFDV by polymerase chain reaction (PCR) amplification of a 310-bp fragment of the small t/large T antigen gene using the primers described by Johne and Müller [11]. The primer sequences were as follows: 5′-CAA GCA TAT GTC CCT TTA TCC C-3′ (Position 4,303–4,324) and 5′-CTG TTT AAG GCC TTC CAA GAT G-3′ (Position 4,612–4,591). The reaction was performed in a total volume of 20 μL that consisted of 1X PCR buffer, 2 mM Mg^{2+}, 200 μM dNTP, 0.2 μM each primer, 2 μL of template, and 1 unit of TaKaRa Ex Taq (TaKaRa Bio Inc., Japan). The following were the PCR amplification conditions: 94°C for 2 min, then 40 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 3 min. The PCR products were separated on 1.5% agarose gels and visualized by UV after staining with EcoDye (BIOFACT Co., Korea).

Complete genome amplification and sequence analysis

A total of four overlapping fragments covered the whole length of the genome amplified using four primer pairs (**Table 1**). The primers were designed based on the sequence of the BFDV-1 strain (accession number AF241168). Each PCR analysis was carried out in a total volume of 50 μL of 1 unit of 2x PCRBIO Ultra Mix Red (PCR Biosystems Inc., UK) 3mM $MgCl_2$, 200 nM of each dNTPs, 200 nM of each primer, and 2 μg of the template DNA. The following were the PCR amplification conditions: 95°C for 5 min, then 40 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min. The PCR products were separated on 1% agarose gels and visualized by staining with EcoDye (BIOFACT CO., Korea).

PCR products were purified using NucleoSpin Gel and PCR Clean-up Kit (Machery Nagel, Germany) according to the manufacturer's instructions. Using the prepared PCR products as templates, sequencing reactions were performed according to the manufacturer's instructions with a BigDye Terminator chemistry (Ver. 3.1) Cycle Sequencing Kit (Applied Biosystems, USA). PCR amplification was performed in 3 μL volumes with 250 ng of templates, 0.5 μL of each primer (3 pmole), 0.87 μL of 5X Sequencing buffer, 1.38 μL of distilled water, and 0.5 μL of BDT v3.1 using a GeneAmp PCR System 9700 (Applied Biosystems). The reaction was performed

Table 1. Nucleotide sequences of primers designed for this study used to amplify and sequence the full-length genome of budgerigar fledgling disease polyomavirus

Primers	Sequence (5′-3′)	Location (regions)	Reference
APV-1F	CTTTTCCTCATCCCCTCCTTTGTC	4853-4876 (t/T-antigen)	Zhuang *et al.*
APV-1R	AGGGGTAGGCGAGTTAGGCTGTGA	810-833 (VP4)	Zhuang *et al.*
APV-1FS[a]	CCTACCCTAAACAGGCCCTT	384-403 (VP4)	This study
APV-2F	AGCACTTCTGTCCCTCCTTC	792-811 (VP4)	This study
APV-2R	CACTGACAGCCTCCCACATA	2176-2195 (VP1)	This study
APV-2FS[a]	AGGAGCGGAATGGTTCTCG	1329-1347 (VP2/VP3)	This study
APV-2RS[a]	GGGGTTTGCTGTTCTATGGT	1759-1778 (VP2/VP3)	This study
APV-3F	GTACCGTGTTACAGCTGTGC	2105-2124 (VP1)	This study
APV-3R	GAACCAGACACAAACCTGCC	3404-3423 (T-antigen)	This study
APV-3FS[a]	GTGTACCCTGCACCCCTAAT	2366-2385 (VP1)	This study
APV-3RS[a]	GGCCCGAAAGTGAAAAGGAG	3097-3116 (T-antigen)	This study
APV-4F	GTTATTACGGAGCGCCTTGG	3181-3200 (T-antigen)	This study
APV-4R	GCTCCTGTGCCTACCTGTAA	4936-4955 (t/T-antigen)	This study
APV-4FS[a]	TTATCGGTGGGCTGTGCTAA	3971-3990 (T-antigen)	This study
APV-4RS[a]	GGGTAGCTCATAGTCGGTGG	4435-4454 (T-antigen)	This study

[a]Primers used for sequencing.

under the conditions of 30 cycles of denaturation at 96°C for 10 s, annealing at 50°C for 5 s, and extension at 60°C for 4 min. PCR products were purified via ethanol precipitation and resolved on the ABI 3730XL DNA Analyzer (Applied Biosystems).

Phylogenetic analysis

The generated sequences were assembled using Geneious Prime software ver. 2022.0.2. (Biomatters Ltd, New Zealand), edited visually and aligned against sequences in GenBank. Phylogenetic trees were generated using the neighbor-joining algorithm applied to Tamura-Nei genetic distances using MEGA11 software. Trees were tested by selecting a bootstrap method with 1,000 bootstrap replicates. For the purpose of genetic comparison, the reference complete genome sequences of BFDV were obtained from the following GenBank accessions: AB453159-AB453165, AB477106, AF241168-AF241170, AY672646, FJ385773, GU452537, KT203762-KT203769, KX008968-KX008969, M20775, MG148345, MH643735, MK061528, MK516256, MN657184, MT119153, and NC_004764.

RESULTS

BFDV detection

The PCR results based on the small t/large T antigen gene marker indicated that BFDV DNA was present in 10 out of 217 screened samples, with an overall prevalence rate of 4.63%. The 217 parrots represent 20 genera, and a total of 10 BFDV-positive parrots were identified in six genera (**Table 2**). Detailed information on the species, age, domestic location, sample types, clinical signs, and gross pathology of the BFDV-positive samples are shown in **Table 3**.

Genetic characterization and phylogenetic analysis of BFDV

Whole-genome sequences were successfully generated from 6 out of 10 strains. The generated full length of the six viral genomes was 4,981 nucleotides. The quality or quantity of DNA preparations from 2 out of 4 strains was not enough for whole-genome sequencing

Table 2. Genera of parrots examined in this study and results of budgerigar fledgling disease polyomavirus examined

Family	Genus	Positive/Examined (positive rate)
Cacatuidae	*Cacatua*	0/7 (0%)
	Eolophus	0/6 (0%)
	Lophochora	0/1 (0%)
	Nymphicus	0/3 (0%)
Psittacidae	*Amazona*	0/19 (0%)
	Ara	0/24 (0%)
	Bolborhynchus	0/1 (0%)
	Deroptyus	0/1 (0%)
	Diopsittaca	0/15 (0%)
	Eclectus	0/18 (0%)
	Guaruba	0/1 (0%)
	Myiopsitta	2/28 (7.14%)
	Pionites	2/10 (20%)
	Pionus	0/2 (0%)
	Poicephalus	0/20 (0%)
	Psittacus	1/26 (3.85%)
	Pyrrhura	1/14 (7.14%)
Psittaculidae	*Agapornis*	0/9 (0%)
	Melopsittacus	1/6 (16.7%)
	Psittacula	3/6 (50%)
Total		10/217 (4.61%)

Table 3. Sample information of budgerigar fledgling disease polyomavirus-positive

Sample No.	Accession No.	Species	Age	Domestic location	Sample type	Clinical signs	Gross pathology
Strain 1	NU[a]	Rose-ringed parakeet (*Psittacula krameri*)	30 d	Chungbuk	Tissue (heart)	Acute death	Petechial hemorrhage on the liver and epicardium
Strain 2	MT981254	Rose-ringed parakeet (*Psittacula krameri*)	25 d	Chungbuk	Tissue (heart)	Acute death	Petechial hemorrhage on the liver and epicardium, retardation of feather growth
Strain 3	NU[b]	Budgerigar (*Melopsittacus undulatus*)	2 mon	Chungbuk	Tissue (heart)	Acute death	Petechial hemorrhage on the epicardium
Strain 4	OM640119	African gray parrot (*Psittacus erithacus*)	2 mon	Busan	Feces	No clinical signs	NP
Strain 5	OM640120	Monk parakeet (*Myiopsitta monachus*)	20 d	Chungnam	Tissue (heart)	Acute death, crop stasis	Petechial hemorrhage on the skin, liver, and epicardium, ascites
Strain 6	NU[a]	Green-cheeked conure (*Pyrrhura molinae*)	2 mon	Chungnam	Feces	Feather dystrophy	NP
Strain 7	OM640121	Rose-ringed parakeet (*Psittacula krameri*)	1 mon	Busan	Tissue (heart)	Acute death	Petechial hemorrhage on the epicardium
Strain 8	OM640122	Black-headed parrot (*Pionites melanocephalus*)	2 mon	Gyeonggi	Tissue (heart)	Acute death	Petechial hemorrhage on the epicardium
Strain 9	OM640123	Black-headed parrot (*Pionites melanocephalus*)	2 mon	Ulsan	Tissue (heart)	Acute death	Petechial hemorrhage on the epicardium
Strain 10	NU[b]	Monk parakeet (*Myiopsitta monachus*)	3 yr	Daejeon	Feces	Multifocal alopecia, self-mutilation	NP

NU, not uploaded; NP, not performed.
[a]Identical sequence, [b]failed to obtain whole genome sequence.

(strains 3 and 10). Strains 1 and 2, and 5 and 6 had 100% identical sequences and were collected from the same domestic locations; thus, the whole-genome sequences of strains 1 and 6 were not submitted to the GenBank. The obtained sequences were submitted to the GenBank database and are available under the following accession numbers: MT981254, OM640119-OM640123.

The phylogenetic analysis reveals that all BFDV strains were largely divided into two groups. MT981254 and OM640119 in this study were grouped into group I with reference strains from Japan (AB453159, AB453160, AB453162, and AB453163), Korea (MK516256), and China (FJ385773). OM640120-OM640123 in this study were grouped into group II with other reference strains from Poland (KT203762-KT203769), Hungary (MN657184), China (AY672646, GU452537, MG148345, MH643735, MK061528, and MT119153), Japan (AB453161, AB453164, AB453165, and AB477106), Portugal (KX008968 and KX008969), USA (AF118150), and Germany (AF241168-AF241170, M20775, and NC_004764). MT981254 was slightly related to other group I strains, with a low bootstrap value (< 70). OM640119 was closely related to MK516256 with a bootstrap value of 100. OM640121 and OM640122 were closely related with a bootstrap value of 99. OM640123 and OM640120 were also slightly related to other group II strains, with a low bootstrap value (< 70) (**Fig. 1**).

To study the mutation of the BFDV strains in this study, based on the phylogenetic tree of the whole genome, 13 reference strains located in group I and group II were selected and compared (**Table 4**). Compared to the consensus sequences, there were 52 variable nucleotide positions were found in the whole genome. OM640120 was the most diverse strain with 15 nucleotide exchanges, followed by OM640119 and OM640123 with 13 nucleotide exchanges, OM640121 with 12 nucleotide exchanges, and MT981254 and OM640122 with 11 nucleotide exchanges. The number of amino acid substitutions ranged from 0.0 to 0.3% in VP1, 0.3 to 0.9% in VP2, 0.4 to 1.3% in VP3, 0.0 to 0.6% in VP4, 0.2 to 0.5% in large T-Ag, and 0.0 to 0.7% in small t-Ag (**Table 5**).

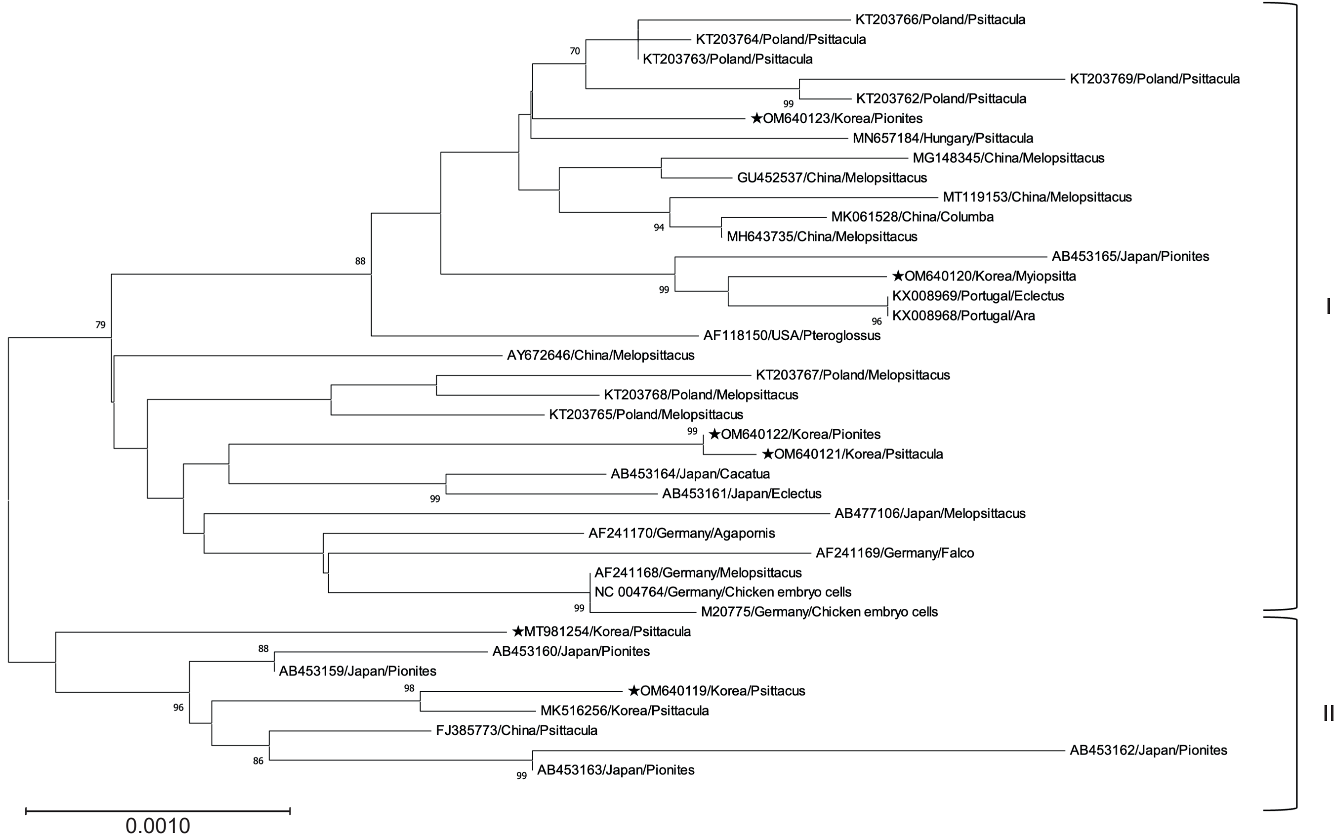

Fig. 1. Estimated phylogenetic trees showing relationship among reference sequences of BFDV. The phylogenetic analysis reveals that all BFDV strains were largely divided into group I and group II. The star (⋆) indicates strains obtained in this study. The bootstrap value on the branch represents the percentage of 1000 bootstrap replicates and only values above 70% are displayed. The neighbor-joining algorithm applied to Tamura-Nei genetic distances as implemented by MEGA11 software.
BFDV, budgerigar fledgling disease polyomavirus.

DISCUSSION

In this study, we described the prevalence and whole-genome sequencing analysis of BFDV in 217 samples from different psittacine species from 2016 to 2019 in South Korea. Totally 10 BFDV-positive samples were collected from the species of Rose-ringed parakeet (*Psittacula krameri*), Budgerigar (*Melopsittacus undulatus*), African gray parrot (*Psittacus erithacus*), Monk parakeet (*Myiopsitta monachus*), Green-cheeked conure (*Pyrrhura molinae*), and Black-headed parrot (*Pionites melanocephalus*). The results indicated that the overall infection rate was 4.63% (10/217). Detections of BFDV were reported in Japan [14], Taiwan [15], Turkey [16], Italy [24], USA [31], and Poland [25] with a prevalence of 2.7%, 15.2%, 48.7%, 0.8%, 6.7%, and 22.2%, respectively. The prevalence of BFDV in South Korea in this study was within the prevalence worldwide (0.8%–48.7%), which was significantly lower than that of Taiwan, Turkey, and Poland, whereas slightly higher than that of Japan and Italy.

The phylogenetic tree based on the complete genome sequences of the BFDV indicated that there was no significant relationship between host adaptability to the strain. MT981254 and OM640121 were collected from the same Rose-ringed parakeet species, whereas these strains were grouped separately. In addition, strains 5 and 6 which were obtained from different species (Green-cheeked conure and Rose-ringed parakeet) had 100% identical genomes.

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Table 4. Point mutations in six strains of budgerigar fledgling disease polyomaviruses compared with consensus

Nucleotide number	Region	Consensus	Nucleotide exchange (amino acid substitutaion)																		
			MT981254	OM640119	OM640120	OM640121	OM640122	OM640123	AB453159	AB453162	AB453163	AB453165	FJ385773	GU452537	KT203762	KT203767	KT203768	KT203769	MH643735	MK516256	MN657184
28	Non-coding	A	T																		
34	Non-coding	T	A											A							
343	VP4 (intron)	C		T						T	T		T							T	
609	VP4 (intron)	T						C						C	C		C	C	C		C
670	VP4	T						C(F→L)		C(F→L)	C(F→L)										
711	VP4	A				C(Q→H)	C(Q→H)									C(Q→H)					
741	VP4	T	C(-)	C(-)					C(-)	C(-)	C(-)		C(-)							C(-)	
831	VP4	C				T(-)															
921	VP4	T		C(-)																	
1301	VP2/VP3	C				T(-)	T(-)														
1513	VP2/VP3	G			A(-)																
1519	VP2/VP3	A		C(-)																C(-)	
1534	VP2/VP3	T			G(-)																
1543	VP2/VP3	A						G(-)													
1591	VP2/VP3	G	T(-)																		
1654	VP2/VP3	G	A(-)																		
1677	VP2/VP3	G		A(R→H)					A(R→H)	A(R→H)	A(R→H)		A(R→H)							A(R→H)	
1694	VP2/VP3	T	C(S→P)	A(S→T)						A(S→T)	A(S→T)		A(S→T)							A(S→T)	C(S→P)
1722	VP2/VP3	A				G(K→R)	G(K→R)									G(K→R)					
1723	VP2/VP3	A						C(K→N)													
1766	VP2/VP3	C		G(Q→E)																	
2151	VP1	A	C(T→P)																		
2177	VP1	G		T(-)											A(-)			A(-)			
2474	VP1	T			C(-)			C(-)				C(-)		C(-)	C(-)			C(-)	C(-)		C(-)
2512	VP1	A		G(K→R)					G(K→R)											G(K→R)	
2525	VP1	T	C(-)																		
2546	VP1	A				T(-)	T(-)														
2558	VP1	G			A(-)			A(-)				A(-)		A(-)	A(-)			A(-)	A(-)		A(-)
2600	VP1	T		C(-)																C(-)	
2663	VP1	A			C(-)			C(-)				C(-)		C(-)	C(-)			C(-)	C(-)		C(-)
2744	VP1	G			A(-)			A(-)				A(-)		A(-)	A(-)			A(-)	A(-)		A(-)
2759	VP1	T			G(-)																
2906	VP1	C			T(-)			T(-)			T(-)		T(-)	T(-)	T(-)			T(-)	T(-)		T(-)
2945	Non-coding	C			A			A				A		A	A			A	A		A
3063	T-antigen	T			G(N→E)	G(N→E)	G(N→E)					G(N→E)				G(N→E)	G(N→E)				
3122	T-antigen	G				T(-)	T(-)														
3224	T-antigen	G	A(-)																		
3242	T-antigen	A						G(-)						G(-)	G(-)			G(-)	G(-)		G(-)

(continued to the next page)

Table 4. (Continued) Point mutations in six strains of budgerigar fledgling disease polyomaviruses compared with consensus

Nucleotide number	Region	Consensus	Nucleotide exchange (amino acid substitutaion)																		
			MT981254	OM640119	OM640120	OM640121	OM640122	OM640123	AB453159	AB453162	AB453163	AB453165	FJ385773	GU452537	KT203762	KT203767	KT203768	KT203769	MH643735	MK516256	MN657184
3413	T-antigen	A				C(-)	C(-)														
3506	T-antigen	A	G(-)	G(-)					G(-)	G(-)	G(-)		G(-)							G(-)	
3663	T-antigen	T	G(D→A)																		
3721	T-antigen	C				A(A→S)	A(A→S)														
3962	T-antigen	C				T(-)	T(-)														
4125	T-antigen	G			A(P→L)	A(P→L)	A(P→L)					A(P→L)				A(P→L)	A(P→L)				
4292	T-antigen	A			G(-)							G(-)									
4430	T-antigen	A		T(-)								-								T(-)	
4769	T/t-antigen	G			T(-)							T(-)									
4811	T/t-antigen	G				A(-)	A(-)									A(-)					
4910	T/t-antigen	A			G(-)																
4948	T/t-antigen	C		A(G→C)																	
4967	T/t-antigen	G						C(-)													
4972	T/t-antigen	A			G(-)			G(-)				G(-)		G(-)	G(-)		G(-)	G(-)	G(-)		G(-)

Minuses indicate the silent mutation positions. Blanks indicate identical nucleotide.

Table 5. Numbers of nucleotide and amino acid substitutions in this study compared with the consensus sequences

Accession No.	bp	No of nucleotide substitutions	VP1 (344 aa)	VP2 (342 aa)	VP3 (236 aa)	VP4 (177 aa)	Larg T (600 aa)	Small t (146 aa)
MT981254	4981	11 (0.2%)	1 (0.3%)	1 (0.3%)	1 (0.4%)		1 (0.2%)	
OM640119	4981	13 (0.3%)	1 (0.3%)	3 (0.9%)	3 (1.3%)		1 (0.2%)	1 (0.7%)
OM640120	4981	15 (0.3%)					2 (0.3%)	
OM640121	4981	12 (0.2%)		1 (0.3%)	1 (0.4%)	1 (0.6%)	3 (0.5%)	
OM640122	4981	11 (0.2%)		1 (0.3%)	1 (0.4%)	1 (0.6%)	3 (0.5%)	
OM640123	4981	13 (0.3%)		1 (0.3%)	1 (0.4%)	1 (0.6%)	3 (0.5%)	
Sequence diversity rate		0.2%–0.3%	0%–0.3%	0.3%–0.9%	0.4%–1.3%	0%–0.6%	0.2%–0.5%	0%–0.7%

Blank cells indicate no predicted amino acid substitutions.
bp, base pairs; aa, amino acid.

According to previous research, a unique amino acid substitution was reported in VP2 (R234H) isolated from Black-headed parrots when compared with the other 12 sequences [32]. However, this mutation was not observed in OM640122 and OM640123 but observed in other psittacine species (FJ385773 and MK516256). According to another previous study, there were different genetic markers were found on the nucleotide locations at 2474, 2558, 2663, 2774, 2906, 2954, and 4972 differences between group I and group II [5]. These findings similarly confirmed the strains obtained in this study. Additionally, there were another two different genetic markers were found. Group I strains contain 741T and 3506A, whereas group II strains contain 741C and 3506G.

Korea plays an important role as a stopover for migratory birds, a breeding place for residents and migratory birds. Furthermore, the popularity of parrots as exotic pets in Korea has increased annually. Unlike mammalian polyomaviruses, avian polyomaviruses have broad host ranges including Psittaciformes, Falconiformes, and Columbiformes [11,12]. Phylogenetic analysis shows that at least two groups of BFDV strains have been introduced into South Korea, and it was found that some nucleotide sequence changes have occurred in domestic strains as well. The results of this study suggest that BFDV has circulated in South Korea. Therefore, advanced epidemiological investigations of BFDV are needed to prevent its transmission to captive parrots and wild birds. It is expected that the total of six whole-genome sequences obtained in this study can be used as additional reference sequences for other researchers.

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