Genetic Analysis of Beak and Feather Disease Virus Derived from a Cockatiel (*Nymphicus hollandicus*) in Japan

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ABSTRACT. Psittacine beak and feather disease (PBFD), which is caused by beak and feather disease virus (BFDV), has been reported in a wide range of psittacine species, except the cockatiel (*Nymphicus hollandicus*), in which PBFD has rarely been reported. We detected BFDV from a case of PBFD in a cockatiel in the present study. The virus was designated CO-JA. The whole genome sequence of CO-JA had from 86 to 98% homology with BFDVs in psittacine species. CO-JA clustered with isolates derived from other cockatoos in phylogenetic analyses based on two major virus proteins. We concluded that genetic data cannot explain the reason why PBFD is rarely found in the cockatiel.

KEY WORDS: beak and feather disease virus, cockatiel, psittacine beak and feather disease, sequence analysis.

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Psittacine beak and feather disease (PBFD) is a lethal disease in psittacine species. Clinical signs of PBFD include chronic, progressive loss of feathers and, in some species, deformities of the beak and claws [7]. Beak and feather disease virus (BFDV), the causative agent of PBFD, belongs to the genus *Circovirus* in the family *Circoviridae* [8]. The BFDV genome is a single-stranded circular DNA of approximately 2 kb that consists of 2 major open reading frames (ORFs), ORF1 and ORF2, encoding the replication-associated protein (Rep) and capsid protein (CP), respectively [1, 5].

PBFD was first described in various species of Australian cockatoos, including sulfur-crested cockatoos, lovebirds, budgerigars and galahs, in 1975 and has since been reported to affect more than 60 psittacine species; it is highly probable that all psittacine species are susceptible [4, 7, 9]. However, BFDV infection has rarely been reported in cockatiels. Here, we report the detection and comparative genomic analyses of a BFDV from PBFD in a cockatiel patient to clarify the reason why PBFD is rarely found in cockatiels.

A 3-year old cockatiel was presented to a veterinary hospital with the complaint of deformities of the feather shaft, dystrophic changes in the primary, secondary, tail and crest feathers and beak abnormality for 2 years (Fig.1). A blood sample was obtained from the bird, and then DNA was extracted from the entire volume (50 μ l) using a SepaGene nucleic acid extraction kit (Sanko Junyaku Co., Tokyo, Japan) to detect pathogens, including BFDV, avian polyomavirus, psittacid herpesvirus and psittacine adenovirus with real-time PCR assays as previous described [2].

BFDV DNA was detected; no DNA of any other pathogenic virus was found. The genome sequence of this BFDV

isolate, which was designated CO-JA, was amplified by PCR using two pairs of primers (Primer 2/PBFDdupR and PBFDdupF/Primer 4) as described previously (Table 1) [6, 10]. The PCR products were directly sequenced by the primer walking method and assembled as described previously [3]. The primers used for walking were designed from published sequences of BFDV in GenBank (Table 1). The sequence read was submitted to DDBJ (Accession number AB514568). The whole genome sequence of CO-JA was 1,991 nucleotides long. The overall percentage of identity ranged from 86% (isolate LK-VIC) and 98% (isolate BFDV-AUS) in the 26 published nucleotide sequences from the public database (Table 2). Sequences derived from cockatiel BFDVs in the other countries (isolates 05–106 and 05–726) were 95% identical to the sequence of CO-JA.

The predicted sizes of Rep and CP were 289 amino acids and 247 amino acids, respectively. The distances of Rep in the CO-JA sequence and other BFDV sequences varied between 1.1% (isolate BFDV-AUS) and 12.1% (isolate LK-VIC) at the amino acid level. Those of CP varied between 3.7% (isolate BFDV-AUS) and 24.3% (isolate LK-VIC). The deduced amino acid sequence of CO-JA showed 97% and 98% homologies in Rep and 93% and 92% homologies in CP to the sequences of cockatiel BFDV isolates 05–106 and 05–726, respectively. As a result of phylogenetic analysis using the neighbor-joining (NJ) method, UPGMA method, maximum parsimony analysis and maximum likelihood approach, the BFDV sequences could be allocated into 11 groups for CP or 10 groups for Rep on the basis of the criteria of >5% divergence, respectively, and the CO-JA sequence clustered with BFDVs isolated from cockatiels and cockatoos (Fig. 2).

Our genetic analyses show that CO-JA is highly similar to other BFDV isolates derived from psittacine species classified into *Psittacinae*. In addition, the clinical symptoms of this bird were similar to those of other psittacine species,

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Fig.1. Appearance of this case. (A) The bird showed loss of flight, crest and tail feathers. (B) It also showed abnormality of the primary and secondary flight feathers.

Table 1. DNA sequences of primers used in this study

Primer	Sequence (5'–3')	Position	Reference
Primer 2	AACCCTACAGACGGCGAG	182–199	[10]
Primer 4	GTCACAGTCCTCCTGTACC	879–898	
PBFDdupF	TTGGGTCCTCCTTGTAGTGGGATC	1371–1394	[6]
PBFDdupR	CAGACGCCGTTTCTCAACCAATAG	1842–1865	
BFDVseq1F	GGTGGCTACCTTATTGCGAG	750–769	This study
BFDVseq2F	TCCTCGCTCACCCCATCAAT	975–994	
BFDVseq3F	GCCAACACTGGTGGTTTGT	1775–1795	
BFDVseq4F	CGGGCATATCTTCGTCTAAT	1920–1939	
BFDVseq1R	CCGTCTGTAGGGTTGTTAAG	176–195	
BFDVseq2R	GAAAGTAGCCTTGCAAATGG	280–299	
BFDVseq3R	CCCTAACAAATCCCAGCACTT	1237–1256	
BFDVseq4R	GGGATTCAAACGCCTCCTCA	1486–1499	

The position for the primers is that of the BFDV-AUS sequence (AF080560).

Table 2. Reference sequences of BFDV used in this study

Isolate	Host bird	Origin	Accession no.
05–106	Cockatiel (<i>Nymphicus hollandicus</i>)	Australia	EF457974
05–726	Cockatiel (<i>Nymphicus hollandicus</i>)	Australia	EF457975
BB-WA	Blue bonnet (<i>Psephotus haematonotus</i>)	Australia	AF311295
LB-WA	Peach-faced lovebird (<i>Agapornis roseicollis</i>)	Australia	AF311296
ELBC-SA	Long-billed corella (<i>Cacatua tenuirostris</i>)	Australia	AF311297
Galah-WA	Galah (<i>Eolophus roseicapillus</i>)	Australia	AF311298
LK-VIC	Rainbow Lorikeet (<i>Trichoglossus haematodus</i>)	Australia	AF311299
MMC-WA	Leadbeater's cockatoo (<i>Cacatua leadbeateri</i>)	Australia	AF311300
SCC-NT	Greater sulfur-crested cockatoo (<i>Cacatua galerita</i>)	Australia	AF311301
SCC1-WA	Greater sulfur-crested cockatoo (<i>Cacatua galerita</i>)	Australia	AF311302
BFDV-AUS	Greater sulfur-crested cockatoo (<i>Cacatua galerita</i>)	Australia	AF080560
WBC1-ZA	White-bellied caique (<i>Pionites leucogaster</i>)	South Africa	AY450434
UC1-ZA	Umbrella cockatoo (<i>Cacatua alba</i>)	South Africa	AY450436
CPA8-ZA	Brown-necked parrot (<i>Poicephalus robustus</i>)	South Africa	AY450437
CPA7-ZA	Brown-necked parrot (<i>Poicephalus robustus</i>)	South Africa	AY450438
RP1-ZA	Ruppell's parrot (<i>Poicephalus rueppellii</i>)	South Africa	AY450439
ARB4-ZA	Red-bellied parrot (<i>Poicephalus rufiventris</i>)	South Africa	AY450440
GJP1-ZA	Red-fronted parrot (<i>Poicephalus gulielmi</i>)	South Africa	AY450441
BCL1-ZAM	Black-cheeked lovebird (<i>Agapornis nigrigenis</i>)	Zambia	AY450442
AFG3-ZA	African grey parrot (<i>Psittacus erithacus</i>)	South Africa	AY450443
PK1-01TX	Ring-necked parakeet (<i>Psittacula krameri</i>)	U.S.A.	AY521234
AR02-IUK	Peach-faced lovebird (<i>Agapornis roseicollis</i>)	United Kingdom	AY521235
PEP01-1POR	African grey parrot (<i>Psittacus erithacus</i>)	Portugal	AY521236
PEG07-1GE	African grey parrot (<i>Psittacus erithacus</i>)	Germany	AY521237
PEU01-1UK	African grey parrot (<i>Psittacus erithacus</i>)	United Kingdom	AY521238
BFDV-USA	Unknown (pooled virus)	U.S.A.	AF071878

Fig. 2. Bootstrapped phylogenetic trees obtained using the neighbor-joining method based on the nucleotide sequences of CP (A) and Rep (B). The numbers at the nodes indicate the bootstrap values. The scale bars indicate the predicted amino acid substitutions in sites.

including cockatiels, as described previously [8, 10]. The origin of CO-JA and other BFDVs isolated in cockatiels might be identical to the BFDVs in cockatoos, which are classified into the pacific species as well as the cockatiel. Worldwide trade of these pacific bird species could cause spread of these related BFDVs to many countries. It is difficult to explain the cause of the rarity of PBFD in the cockatiel from a genetic point of view. Although no serological approaches were attempted in this study, Shearer *et al.* reported that the BFDVs from cockatiels are serologically different from other BFDVs [9]. In addition, other factors, including the host immune system, could be related to the rare incidence. In conclusion, it is necessary to focus on not only the viral factors but also the host immune system in order to understand BFDV infection in cockatiels.

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