Molecular Characterization of Budgerigar Fledgling Disease Virus SC-YB19, with an 18-Nucleotides Deletion, in China

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Research article

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Abstract

Background: Budgerigar fledgling disease virus (BFDV) poses a serious threat to the Chinese psittacine industry, causing enormous economic losses. This study aims to reveal the etiological role of BFDV and evaluate the molecular characterization.

Results: We report on BFDV, designated SC-YB19, which had an 18-nucleotide (nt) deletion in the enhancer region, corresponding to the sequence position 164–181 nt, when compared with other BFDV strains. Sequence analyses suggested that 19 nucleotide substitutions were identified with the domestic strains, APV7 and AF118150. Phylogenetic analysis indicated that SC-YB19, along with three domestic strains, formed a unique cluster, and were closely related to Polish isolates.

Conclusion: Taken together, these results demonstrate that a BFDV genotype variation was co-circulating in China, and provide important insights on evolution of BFDV.

Background

Budgerigar fledgling disease virus (BFDV) is a polyomavirus, and is the causative agent of budgerigar fledgling disease, which is an important viral disease of budgerigars (Melopsittacus undulatus). The disease was first reported in 1981, with typical symptoms including abdominal distention, lack of down feathers on the back and abdomen, subcutaneous hemorrhage of nestling budgerigars, and acute death [1-3]. Polyomavirus has a wide host range, with novel polyomaviruses identified in vertebrate hosts including humans [4, 5], bats [6, 7], non-human primates [8], and horses [9]. In 1994, the first BFDV report was made in Hubei province, China, however the virus still causes sporadic infections across China, and inflicts significant losses to the budgerigar breeding industry [10].

BFDV, also called avian polyomavirus (APV), was identified as the first non-mammalian member of the genus polyomavirus [11, 12]. BFDV is a circular, double-stranded molecule with a genome of 4981 nt, which can be divided into an early and a late region. The early region codes for two nonstructural proteins, the large T and the small t antigen. The late region contains four structural proteins, VP1, VP2, VP3, and VP4 [13]. Similarly, the genome also contains four regulatory elements, a promoter, polyadenylation signals, origin of DNA replication and enhancer [14].

In the present study, we report a BFD infection in a budgerigar from Sichuan province for the first time. To better understand the molecular characteristics of this strain, sequencing analysis and the phylogenetic tree was constructed based on its complete genome, which may assist in elucidating the genetic evolution of BFDV in China.

Methods

Ethics statement

No animals were sacrificed specifically for this study.

Clinical case

During the spring of 2019, there was a budgerigar dead in the farm. Samples of hearts, livers, lungs and faeces were collected from the dead budgerigars. Each sample was subjected to grind and centrifuged at 8,000 rpm for 10 min. The DNA was extracted from supernatant, then identified with the primers [15].

Sequence alignment and phylogenetic analysis

Multiple sequence alignments were conducted using ClustalW in MEGA 6.0. A phylogenetic tree, based on complete sequences, was constructed using the neighbor-joining (NJ) method using MEGA software (version 4.0). Bootstrap values were estimated for 1,000 replicates. The sequences obtained in this study were assembled and submitted to GenBank under the accession number MT119153.

Results

Sequence analysis of SC-YB19

PCR assay was carried out to confirm that BFDV was found in the samples. The strain, designate SC-YB19, was 4963 nucleotides (nt) in length. When compared with APV7, eighteen nt deletion was identified in enhancer (Figure 1A and 1B). Furthermore, nucleotide substitutions were observed at 19 loci in the SC-YB19, AF118150, APV-7, AF241168-70, M20775 and NC004764 (Figure 1C). Three nucleotide substitutions were found in VP4 (position 821), VP1 (position 2383) and the T-antigen (position 3517), and were unique to SC-YB19 (Table 2).

Phylogenetic analysis

Phylogenetic analysis of the complete sequence showed that BFDV strains could be divided into three groups (Figure 1D). The SC-YB19 had a close relationship with domestic WF-GM01, SD18, APV-P strains and is more distant evolutionarily from the AY672646 strains group and strain QDJM-01.

Discussion

In 2019, a privately owned two-week-old male budgerigar was submitted for laboratory investigation, after its untimely death. A necropsy revealed subcutaneous hemorrhage, ascites and hydropericardium. Multiple areas of hemorrhage were observed in the liver. Tissue samples from the liver and lungs were positive for BFDV. No other viruses were identified in samples (data not shown).

To analyze genomic characteristics, PCR assay was employed to amplify and sequence the complete viral genome (termed SC-YB19), which was 4963 nt. The BFDV genome incorporated an early region, a late region, promoter, polyadenylation signals, origin of DNA replication and enhancers. The enhancers consisted of two parts at 80–126 nt and 131-178 nt, when compared with APV7.

The genetic diversity of SC-YB19 and APV isolates was investigated using multiple alignment analyses of whole genome sequences from APV1–7 isolates, which were isolated from Japan between 2003 and 2006. There were common and unique features between SC-YB19 and BFDV strains. Firstly, an 18-nt deletion was detected in the latter part of the enhancer section in SC-YB19, but not in the other BFDV isolates (Fig. 1A and B). Deletions in this region have not been previously described for BFDV, and may be associated with transcription activities [14]. Further experiments will be required to confirm the biological effects of this sequence deletion. In addition, nucleotide substitutions were found in the SC-YB19, AF118150, APV-7, AF241168-70, M20775 and NC004764 (Table 2). Notably, no amino acid substitutions were observed (Table 1). These data indicate that SC-YB19 sequences were consistent with at least two out of five domestic strains in terms of mutation positions, but distinct to foreign strains (Fig. 1C). These observations further suggest that five domestic strains showed genetic diversity and underwent evolution at some point in the past.

Phylogenetic analyses based on complete sequences, suggested that SC-YB19, along with the domestic strains, WF-GM01, SD18 and APV-P formed a unique cluster and were closely related to Polish virus isolates. However, QD-JM01 was located in a cluster of APV1, APV2, APV4 and APV5 strains, which were isolated from the Japanese black-headed caique (Pionites melanocephalus). AY672646 was distinct with five domestic strains and did not belong to any cluster (Fig. 1D). Our data showed that different BFDV genotypes were co-circulating in China.

Conclusion

We report for the first time a novel mutation in BFDV, in mainland China. The strain, along with five domestic strains forms a unique cluster. We theorize these viruses were co-circulating in China, and underwent a similar evolution.

Abbreviations

Budgerigar fledgling disease virus (BFDV); avian polyomavirus (APV); neighbor-joining (NJ); nucleotides (nt).

Declarations

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Authors' contribution

HXL designed the study, HXL, YCM & TZG performed the experiments, TZG and YCM draft the manuscript. CF and HXL revise the draft. All authors revised and approved the paper for publication.

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Availability of data and materials

DNA sequences obtained in this study have been submitted to GenBank database (accession number: MT119153).

Ethics approval and consent to participate

The present study was approved by Animal Ethics Committee of Yibin University, Yibin, China, according to the OIE standards for use of animals in research and education. Tick samples were collected with permission from the farmer. Each of the farmer wrote consent and consented to this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1: Source of Budgerigar fledgling disease polyomavirus (BFDV) sequences used in the experiment

Name	Genbank	Country
SC-YB1		CHN
BFDV1	AF241168	GER
BFDV4	AF241169	GER
BFDV5	AF241170	GER
GFM-1	AB477106	JPN
BFDV	AF118150	USA
APV1-7	AB453159–AB453166	JPN
PLYGEN	M20775	GER
/	NC004764	GER
WF-GM01	GU452537	CHN
/	AY672746	CHN
SD18	MH643735	CHN
QDJM-01	FJ385773	CHN
PL830X	KT203762	POL
PL1220B	KT203766	POL
PT25528	KX008968	POR
PT919	KX008969	POL

Table 2: Point mutations in seven strains of BFDVs compared with SC-YB19.

Nucleotide number	Region	SC-YB19	Nucleotide exchange compared with SC-YB19 (amino acid substitution compared with predicted amino acid sequence of SC-YB19)					
			SD18	WF-GM01	QDJM01	AY672646	APV7	AF241168
386	VP4(inton)	G	G	G	G	G	G	C
387	VP4(inton)	C	C	C	C	C	C	G
623	VP4(inton)	C	C	C	T	T	T	T
821	VP4	C(123T)	T(123T)	T(123T)	T(123T)	T(123T)	T(123T)	T(123T)
1652	VP2/VP3	G(115G)	G(115G)	G(115G)	G(115G)	G(115G)	G(115G)	T(115A)
2383	VP1	T(157S)	A(157S)	A(157S)	A(157S)	A(157S)	A(157S)	A(157S)
2488	VP1	C(192G)	C(192G)	C(192G)	T(192G)	T(192G)	C(192G)	T(192G)
2572	VP1	A(220E)	A(220E)	A(220E)	G(220E)	G(220E)	A(220E)	G(220E)
2677	VP1	C(255A)	C(255A)	C(255A)	A(255A)	A(255A)	C(255A)	A(255A)
2758	VP1	A(282R)	A(282R)	A(282R)	G(282R)	G(282R)	A(282R)	G(282R)
2920	VP1	T(336D)	T(336D)	T(336D)	C(336D)	C(336D)	T(336D)	C(336D)
2959	Non-coding region	A	A	A	C	C	A	C
3256	T-antigen	G(515T)	G(515T)	G(515T)	A(515T)	A(515T)	A(515T)	A(515T)
3457	T-antigen	C(448K)	C(448K)	T(448K)	T(448K)	T(448K)	T(448K)	T(448K)
3517	T-antigen	G(428R)	T(428R)	T(428R)	T(428R)	T(428R)	T(428R)	T(428R)
3657	T-antigen	T(382K)	T(382K)	G(382Q)	G(382Q)	G(382Q)	G(382Q)	G(382Q)
3739	T-antigen	G(354T)	G(354G)	G(354G)	A(354T)	A(354T)	A(354T)	A(354T)
4139	T-antigen	G(221P)	G(221P)	G(221P)	G(221P)	A(221L)	A(221L)	A(221L)
4986	T/t-antigen	G(4L)	G(4L)	G(4L)	A(4L)	G(4L)	G(4L)	A(4L)

Highlights indicate the mutant position.

Figures

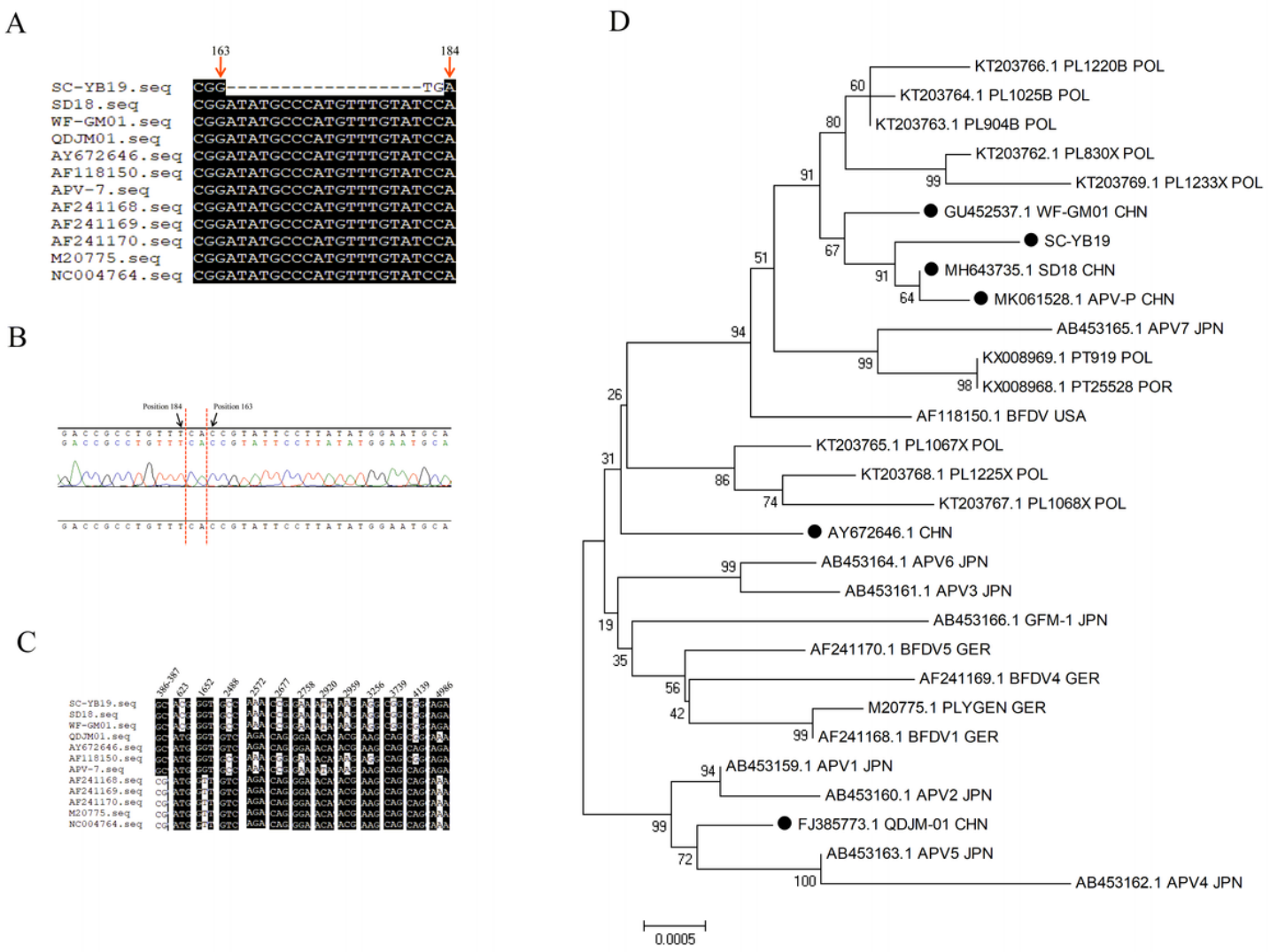

Figure 1

(A) Multiple sequence alignment of position 161–184 nt in BFDV strains. (B) Sequencing analyses. (C) Multiple sequence alignment of complete sequences in BFDV strains. (D) Phylogenetic analysis of complete SC-YB19 strain sequences and related whole genome strains from GenBank. Neighbor-joining was used to construct the phylogenetic tree, with bootstrap values of 1,000 replicates shown at the branches. The scale bar represents the p-distance.