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Genomic and pathogenic analysis of a Muscovy duck parvovirus strain causing short beak and dwarfism syndrome without tongue protrusion

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ABSTRACT

In 2008, clinical cases of short beak and dwarfism syndrome (SBDS) caused by Muscovy duck parvovirus (MDPV) infection were found in mule duck and Taiwan white duck farms in Fujian, China. A MDPV LH strain causing duck SBDS without tongue protrusion was isolated in this study. Phylogenetic analysis show that the MDPV LH strain was clustered together with other MDPV strains, but divergent from GPV isolates. Two major fragment deletions were found in the inverted terminal repeats (ITR) of MDPV LH similar to the ones in the ITR of MDPV GX5, YY and SAAS-SHNH strains. To investigate the pathogenicity of the MDPV LH strain, virus infection of young mule ducks was performed. The infected ducks showed SBDS symptoms including retard growth and shorten beaks without tongue protrusion. Atrophy of thymus, spleen and bursa of Fabricius was identified in the infected ducks. The results show that MDPV LH strain is moderately pathogenic to mule duck, leading to occurrence of SBDS. As far as we know, it is the first study showing that SBDS without tongue protrusion, and atrophy of thymus, spleen and bursa of Fabricius possibly associated with immunosuppression were found in the MDPV-infected ducks. The established duck-MDPV-SBDS system will help us to further work on the virus pathogenesis and develop efficacious vaccine against MDPV infection.

1. Introduction

Muscovy duckling parvovirosis is an acute viral infectious disease caused by Muscovy duck parvovirus (MDPV). The major pathological changes and clinical symptoms of this disease include diarrhea, panting, weakened foot, bleeding or white necrotic spots in pancreas, and duodenal mucosal bleeding (Glávits et al., 2005). This disease is also called "Muscovy 3-week disease" because MDPV mainly infects 3-week-old Muscovy ducks. The morbidity and mortality caused by Muscovy duckling parvovirosis are 27%–62% and 22%–43% respectively (Lin et al., 1991). Although infected ducks may be cured, most healed ducks cannot further grow. As a result, Muscovy duckling parvovirosis results in great economic loss in duck industry.

In 1985, a disease with major symptoms include diarrhea, panting, and weakened feet was observed among Muscovy ducklings in regional duck farms and hatcheries in Fujian Province, China. In 1988, the etiological agent was identified as MDPV, a new member of the genus parvovirus (Lin et al., 1991; Liu et al., 1993). In 1991, Muscovy duckling diseases caused by MDPV were found in Taiwan and in many other provinces in China (Chang et al., 2000). A new Muscovy duckling disease has caused 80% mortality in the western region of France, and the clinical symptoms and pathological changes are similar to those of Derzsy's disease (goose parvovirus) (Gallreculé and Jestin, 1994; Jestin et al., 1991). The MDPV causing Muscovy duckling disease was also reported in the United States, Japan, Thailand and Hungary, Poland (Glávits et al., 2005; Sirivan et al., 1998; Takehara et al., 1994; Woolcock et al., 2000; Woźniakowski et al., 2012).

A duck disease characterized with SBDS was observed in mule ducks in the southwest region of France in the early 1970s. The pathogen was speculated to be a goose parvovirus (GPV), but it was not isolated (Palya et al., 2009). The causative agent of SBDS had not been isolated and identified as GPV until the end of the 1990s. Moreover, Vilmos experimentally reproduced SBDS in mule ducks with isolated GPV strain (Sirivan et al., 1998). A novel moderately pathogenic GPV-related parvovirus (N-GPV) had been reported in commercial Cherry Valley duck flocks and mule ducks in several provinces of China. It consistently showed the similar symptoms of SBDS with swollen tongue, watery diarrhea, shorter tibia, mild hepatic atrophy swelling,

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Table 1
Information about reference viruses.

type	GenBank No.	Strains	Isolation time	Host	Regions
MDPV	U22967	FM	1994	*Cairina moschata*	Hungary
	KC171936	SAAS-SHNH	2012	Muscovy duck	China
	JF926697	P	1988	Muscovy duck	Fujian, China
	KX000918	YY	2000	Muscovy duck	China
	KU844282	P1	Not available	Muscovy duck	Fujian, China
	KT865605	FZ91–30	1991	Muscovy duck	Fujian, China
	KM093740	GX5	2011	Muscovy duck	China
GPV	U25749	B	1960s	Goose	Hungary
	KC178571	Y	2011	Muscovy duck	China
	KC184133	E	2011	Goose	China
	KM272560	LH	2012	Goose	China
	EU583390	82-0321	1982	Goose	Taiwan, China
	JF333590	SH	2009	*Anser anser*	China
	KC478066	SHFX1201	2012	swan	China
	KC996730	YZ99–6	1999	*Anser anser*	China
	HQ891825	GDaGPV	1978	goose	China
	EU583389	82–0321 V	1982	goose	China
	EU583392	VG32/1	Not available	goose	Hungary
	KC996729	SYG61v	1961	goose	China
	AY496906	D146/02	2004	mule duck	France
N-GPV	KU844283	M15	2015	Cherry Valley duck	Fujian,China
	KT751090	QH15	2015	Not available	China
	KT343253	SDLC01	2015	Cherry Valley duck	China

hemorrhage in the thymus, moderate morbidity rates and low mortality rates (Chen et al., 2015; Chen et al., 2016).

In 2008, the SBDS was found in young mule ducks and Taiwan white ducks in Fujian, China. The diseased ducks also presented weakened foot, fractured tibias, and shorten beaks with 30% reduction in size. Although this disease didn't cause high mortality, 60% of the infected ducks were clinically diseased. Most of them could not further grow and showed obvious retard growth. In this study, a novel MDPV strain causing SBDS without tongue protrusion was isolated and designated MDPV LH. Complete genomic sequencing of the MDPV LH strain and phylogenetic analysis with a number of duck and goose parvovirus isolates and vaccine strains were performed. The pathogenicity of MDPV LH strain in ducks was determined. The results obtained will greatly help elucidate the pathogenesis of MDPV and develop next generation of vaccine against newly emergent virus variant infection.

2. Materials and methods

2.1. Ethical statement

The animal protocol (FAAS-AEC-2010-0311) used in this study was approved by the Research Ethics Committee of Fujian Academy of Agricultural Science. All birds' experimental procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals approved by the State Council of China. All efforts were made to minimize suffering. The diseased ducks were euthanized by carbon dioxide before sample collection. There are no specific permissions required for these locations or activities and not endangered or protected species involved in these locations.

2.2. Virus isolation and characterization

In 2008, clinical samples were collected from infected mule ducks characterized with SBDS in Fujian, China. Livers and spleens were collected, homogenized and suspended in Phosphate Buffered Saline (PBS). After centrifugation at 12000 × *g* for 1 min at 4 °C, the supernatants were filtered through 0.22 μm filters and then used for DNA extraction and virus isolation. The filtered supernatants were inoculated into the chorioallantoic cavity of 10-day-old duck embryonic eggs (0.2 mL/egg). The allantoic fluid and embryo were collected from dead eggs from 2 days post infection (dpi) to 10 dpi. The extracted DNA from the allantoic fluid and embryo homogenates were used for detection of parvovirus. Finally, the virus strain was harvested after three rounds of plaque purification in the primary cell cultures of duck embryo.

To analyze the virus particles under electron microscope, the virus-containing allantoic fluid was centrifuged at 10,000 × *g* for 10 min, followed by two steps of centrifugation including at 20,000 × *g* for 2 h and at 40,000 × *g* for 4 h. The sediments from the last centrifugation were suspended in PBS and sprayed onto 200-mesh carbon-formvar coated copper grid. Virus particles were examined and photographed using an electron microscope.

2.3. Viral DNA extraction and genome sequencing

DNA was extracted by using QIAamp DNA mini kit (Qiagen) according to the manufacturer's instruction. A 493-base pair (bp) (2562 to 3054) fragment of VP1 was amplified by PCR using specific primers (forward: 5′-AGAAAACCCCAACGAAAAGA-3′ and reverse: 5′-CTCCGCTTCCTCCCTCTG-3′) (Palya et al., 2009). The amplified PCR products were purified and sequenced. After the embryonic egg-derived fluid was identified positive for MDPV, complete virus genome sequencing analysis was then performed with primers according to Wan et al. (2015). PCR condition was generally as follows: an initial denaturation step at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 5 min, annealing at 53 °C for 35 s, extension at 72 °C for 75 s, and a final extension at 72 °C for 10 min. The amplified PCR products were purified using gel extraction kit (Omega Bio−Tek, GA, USA) and ligated into a pBackZero8-T vector (Takara, Dalian, China). Five positive clones were randomly selected and sequenced. Full-length virus genome was assembled using the Lasergene 7.1.0 software package (DNASTAR Inc).

2.4. Sequence analysis

The molecular characteristics and homology of nucleotide sequences of the entire viral genome and the deduced amino acids were analyzed using the MegAlign tool included in the Lasergene software package. Genetic distance was calculated using the DNASIST program (Kimura two-parameter method) included in the PHYLIP software