

NUCLEOTIDE SEQUENCE AND PHYLOGENETIC ANALYSIS OF GOOSE PARVOVIRUS DETECTED FROM PEKIN DUCK

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ABSTRACT. Goose Parvovirus (GPV) also known as Derzy's disease is an infectious viral disease of waterfowl which causes serious economic loss in industrial production of geese and Muscovy ducks. In year 2014, Derzy's disease was detected in Pekin ducks from Sarawak. The affected farm recorded up to 50% mortality, affecting only young ducklings (starting at the age of 3 weeks). Polymerase chain reaction (PCR) from liver samples were performed based on partial region of VP3 gene of GPV, generated amplicon of 801 bp. Sequence analysis showed that the isolate shared 99% sequence similarity with goose parvovirus strain YBLJ and YZYZZ20130304 from China. Phylogeny based on VP3 showed that this isolate is grouped under Asian strains. This is the first report of GPV in Malaysia focusing on the molecular analysis. Notably, this study revealed that GPV not only can be detected from goose and Muscovy but also from Pekin duck.

Keywords: goose parvovirus, Derzy's, Sarawak, Pekin ducks, China

INTRODUCTION

Waterfowl parvoviruses cause high morbidity and mortality in goslings and Muscovy ducklings, with mortality rates between 10% and 80% and even up to 95% (Chen *et al.*, 2015). Studies have shown that waterfowl parvoviruses can be divided into two groups: the goose parvovirus (GPV)-related group, and the Muscovy duck parvovirus (MDPV)-related group (Wang *et al.*, 2013).

This disease is also known as Derzy's disease which is characterized by anorexia, prostration, watery diarrhea, enteric symptoms, and death. Surviving young birds and infected older birds show degenerative skeletal muscle myopathy and growth retardation (Chen *et al.*, 2015). This viral disease has caused high mortality and morbidity in geese and ducks (Shien *et al.*, 2008; Barnes, 1997).

Waterfowl parvoviruses are members of the genus Anseriform dependoparvovirus 1 in the family Parvoviridae and contain a linear, single-stranded DNA genome of about 5 kb in length. The genome contains two major open reading frames (ORFs): the left ORF that encodes for the regulatory (Rep) protein, and the right ORF that

encodes for three capsid proteins, VP1, VP2, and VP3. VP3 is the most abundant and can induce neutralizing antibody (Woźniakowski *et al.*, 2009). Many studies of phylogenetic analysis of GPV have been conducted based on VP3 gene (Deemagarn *et al.*, 2015).

The history of GPV started in China in 1956 where it caused serious disease of goslings as reported by Wang in 2005. In the 1960s, several countries in Europe were reported to face a similar disease but they referred this disease as “goose influenza”. It was not until 1971 that Schettler (1979) confirmed that the disease was caused by a parvovirus. In 1979, it was recommended for the disease to be named as goose parvovirus. A new parvovirus, Muscovy duck parvovirus (MDPV), was discovered in 1989 isolated from the Muscovy duck. GPV and MDPV differ in host ranges, antigenicity and nucleotide sequences. GPV can cause highly contagious and fatal disease in goslings and Muscovy ducklings; whereas MDPV only cause disease with Muscovy ducklings (Tatarakis *et al.*, 2004).

In this study, PCR was carried out on the VP3 gene of GPV from Pekin Duck in Sarawak. Further analysis was conducted on the nucleotide sequence and characterization of this isolate with other 34 GPV strains from different parts of the world using a phylogenetic approach.

METHODOLOGY

In 2014, liver and spleen samples were sent to the Veterinary Research Institute. These samples were collected from weak Pekin ducklings in Sarawak suspected to be infected by Derzy's Disease. These weak ducklings (aged of 3 weeks) were reported to have high mortality and morbidity up to 50%, respectively.

DNA Extraction and Polymerase Chain Reaction

Viral DNA was extracted from the liver samples using DNeasy Blood and Tissue Kit (Qiagen, USA) according to manufacturer's instructions. Subsequent PCR was carried out using GoTaq® Green Master Mix (Promega, USA). A pair of primers AL18F2 (5'- CCG GGT TGC AGG AGG TAC-3') and AL18R2 (5'-AGC TAC AAC AAC CAC ATC-3') from Sirivan 1998 was used to amplify VP3 gene to generate 806 bp amplicon. After completing the PCR, the reaction mixture was loaded into 1.5% agarose gel containing SyBr Safe (Invitrogen, USA) for quality check through electrophoresis and visualized by UV transilluminator.

Nucleotide Sequencing

PCR products were cut from agarose gel and purified using QIAQuick Gel Extraction Kit (Qiagen) prior to sequencing. Sequencing was performed by First Base Laboratories (First Base, Malaysia). The primers used

for sequence analysis were the same as those used for PCR amplification. The sequences were assembled using Seqman (DNASTar Lasergene, USA).

Nucleotide sequences were then analyzed using BioEdit program version 7.2.5 and multiple sequence alignment was performed with Clustal W (Hall, 1999). For phylogenetic analysis, the analysis included other GPV sequences obtained from GenBank (GenBank, NCBI). Phylogenetic tree was constructed with MEGA v6.06 using neighbour joining Kimura 2 parameter model with 1,000 bootstrapped replications (Tamura *et al.*, 2011) and rooted with VP3 sequence of MDPV strain FM (Gen Accession No. X75093) as showed in previous studies (Deemagarn *et al.*, 2015, Chen *et al.*, 2009). In this study, the phylogenetic analysis of

the GPV isolates was generated based on the region of 495 bp long of VP3 gene that the isolate and other references had in common; from nucleotide 163 to 657.

RESULT

Using primer of AL18F2 and AL18R2, VP3 gene was successfully amplified at the expected size of 801 bp. (Figure 1).

High similarities of nucleotide sequences between the Malaysian isolate 11936/2014 and China strains (YBLJ and YZZ20130304) were observed with similarities of 99.7% (Table 1).

Though they have some differences in nucleic acid, similarities of VP3 amino acid sequences showed that all strains are 100% identical to the Malaysian isolate 11936/2014 except for MDPV with 93.3%

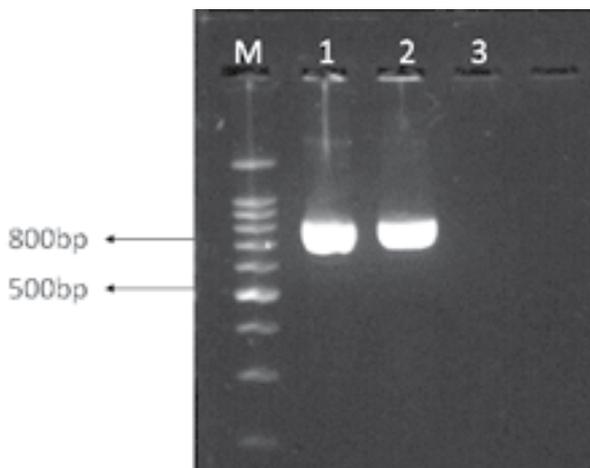


Figure 1. Agarose gel electrophoresis of PCR product of Derzy's disease (GPV) of 11936/2014 that encodes VP3 gene with amplicon 801 bp. Lane M: Molecular marker 100 bp; Lane 1:11936/2014; Lane 2: Positive PCR control of Derzy's Disease (10244/2003); Lane 3: Negative PCR control.

Table 1. Similarity analysis of the nucleotide sequences of partial VP3 gene between Malaysian isolate 11936/2014 and other strains including pathogenic (P) and vaccine (V) strains. Values in the upper right and lower left indicate percent similarities of the nucleotide and deduced amino acid sequences, respectively.

		Percent similarity of nucleotide sequences (%)										
		1	2	3	4	5	6	7	8	9	10	
11936/2014- Malaysia	1		99.7	99.7	97.7	97.7	97.1	98.3	96.7	96.3	83	1
YZYZ20130304-2013-China	2	100		100	97.9	97.9	97.3	98.5	96.9	96.5	82.8	2
YBLJ China	3	100	100		97.9	97.9	97.3	98.5	96.9	96.5	82.8	3
Thailand/2012 (P)	4	100	100	100		100	98.9	96.7	95.3	94.9	82.4	4
SHFX1201-China (P)	5	100	100	100	100		98.9	96.7	95.3	94.9	82.4	5
98-1218-Taiwan (P)	6	100	100	100	100	100		96.1	94.7	94.5	82.6	6
B42-Hungary (V)	7	100	100	100	100	100	100		97.3	96.9	83.4	7
Hoekstra-France (V)	8	100	100	100	100	100	100	100		98.7	83.4	8
SDLC01-2015-China	9	100	100	100	100	100	100	100	100		84	9
MDPV-Hungary (P)	10	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3	93.3		10
		Percent similarity of the amino acid sequences (%)										

similarities. This is based on 165 amino acid (positions 55 to 219 aa) of VP3 amino acid sequences (Table 1).

The phylogenetic tree constructed based on VP3 nucleotide sequences divided the isolates into 5 groups, including Asian, French, virulent Hungarian, low virulent Hungarian and Polish strains. Based on phylogenetic analysis of the VP3 gene sequences, Malaysian isolate 11936/2014 was found to be closely related to strains from Taiwan, China and Thailand which were grouped as Asian strains (Figure 2).

Malaysian isolate 11936/2014 was grouped in Asian strains but was placed in a cluster together with strains from China (YBLJ and YZZ20130304). Although, the Thai isolate (T-2012) was also grouped as Asian strains, it is more closely related to

isolates from Taiwan. For isolate SDLC01 isolated from Cherry Valley, though it was from China, but was clustered together in French strains.

Additionally, all vaccines strains were grouped in European strains (French, Hungarian and Polish). When nucleotide sequence of the Malaysian isolate 11936/2014 was compared with vaccine strains, they had similarities in the range of 96.7% to 98.3% while for amino acid similarities, they were identical.

DISCUSSION

Based on the VP3 sequence analysis, it can be concluded that the Derzy’s disease in Pekin ducklings in this study was caused by GPV. This isolate of GPV (11936/2014)

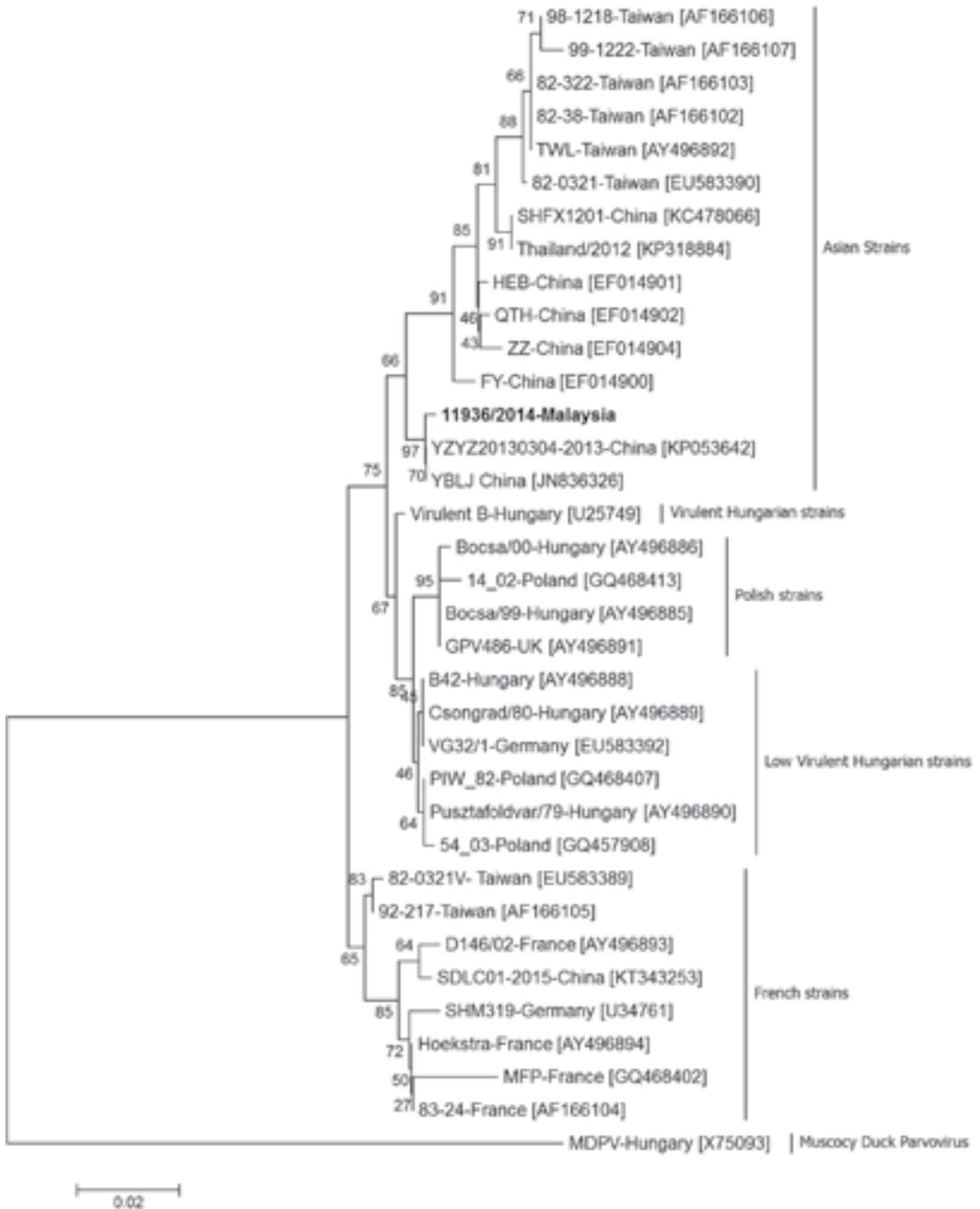


Figure 2. Phylogenetic tree constructed based on 495-bp long nucleotide sequences of VP3 gene of Malaysia GPV isolate and other GPV isolates from several countries showing their relationship among GPV strains. The GPV isolate obtained in the present study are in bold. Accession numbers of the sequences from GenBank are shown in parenthesis.

caused the disease in Pekin ducklings at the age of 3 weeks and is similar with studies reported by Ji *et al.* (2010) and Chang *et al.*, (2000).

Previously, it has been reported that GPV is able to cause disease in geese and Muscovy ducks not only in Taiwan but Thailand, China, Hungary, France, Germany and Poland (Chang *et al.*, 2000; Deemagarn *et al.*, 2015; Shien *et al.*, 2008; Tatar-kis *et al.*, 2004; Wozniakowski *et al.*, 2012).

In 2009, Palya *et al.*, reported that GPV has been isolated from mule duck. Mule duck is a sterile, intergenetic cross of Pekin and Muscovy ducks. Interestingly, it was also reported by Chen *et al.* (2015) that newly emerged duck parvovirus which causes beak atrophy and dwarfism syndrome (BADs) in Cherry Valley ducks has appeared in northern China. According to Newton (2012), Cherry Valley ducks were called Pekin duck in the 1950s before it spread to many parts of the world (including Europe). The rise of the modern duck industry in the UK started in Lincolnshire in 1959 with the establishment of the Cherry Valley Company. It is from that base that most of the Pekin breeding ducks are now referred to as Cherry Valley ducks. Therefore 11936/2014 can be considered the second reported case on GPV-causing disease in Pekin ducks.

Isolate 11936/2014 shared high percent similarities of nucleotide sequences with YBLJ and YZYZZ20130304 strains from China. This indicates that the

11936/2014 isolate originated from China, as YBLJ and YZYZZ20130304 strains were reported to be isolated in year 2006 and 2013 respectively (Shao, 2014; Gao *et al.*, 2011).

SLDC01 and 11936/14 are considered from the same host, Pekin duck, based on sequence and phylogenetic results, where both showed 96% similarities. However, both were grouped into different clusters where SLD01 is a French strain and 11936/2014 is an Asian strain. In addition, SLDC01 is considered as a novel goose parvovirus (N-GPV) because this isolate was distinct from all GPV and MDPV isolates based on phylogenetic analysis of Rep and VP1 genes (Chen *et al.*, 2015), further supporting this observation.

In order to control and prevent this disease, treatment of young animals with hyperimmune serum or vaccination of growers and breeders to the progeny is recommended. The live attenuated or inactivated vaccines contain whole or recombinant antigens that aim to provide high levels of maternally derived antibody (Tatar-kis *et al.*, 2004). At present, two types of inactivated vaccines have been used in Malaysia to control Derzy's disease that are from GM and Hoekstra strains. Both vaccines are manufactured by Merial, France (Anon, 2015). Based on this study, it is recommended to not only vaccinate geese and Muscovy ducks but also Pekin (Cherry Valley ducks) as this breed is also susceptible to Derzy's disease.

In conclusion, this is the first GPV reported on Pekin duck in Malaysia

focusing on a molecular approach. It is recommended that more studies should be conducted on this isolate from other genes like Rep and VP1 so that it will further contribute to the understanding of the epidemiology and control of this disease in Malaysia.

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