Veterinary Microbiology 210 (2017) 17–23

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Pathogenicity of Pekin duck- and goose-origin parvoviruses in Pekin ducklings

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ARTICLE INFO

Keywords:
Short beak and dwarfism syndrome
Derzsy's disease
Goose parvovirus
Pekin duck

ABSTRACT

Goose parvovirus (GPV) usually affects goslings and Muscovy ducks but not Pekin ducks. Earlier works showed that a variant GPV can cause short beak and dwarfism syndrome (SBDS) in Pekin ducks. Here, we investigated the pathogenicity of a variant GPV of Pekin duck-origin (JS1) and a classical GPV of goose-origin (H) in Pekin ducklings. Following intramuscular infection at two days of age, both JS1 and H strains influenced weight gain and development of beaks and bones of wings and legs, and caused microscopic lesions of internal organs of ducks. However, the clinical signs typical of SBDS could only be replicated with the JS1 isolate. The findings suggest that both variant and classical GPVs are pathogenic for Pekin ducklings, while the former is more virulent than the latter. Using a quantitative real-time PCR assay, high levels of viral load were detected from bloods, internal organs, leg muscles, and ileac contents in JS1- and H-infected ducks from 6 h to 35 days postinfection (DPI). Using a GPV VP3-based ELISA, antibodies in sera of JS1- and H-infected ducks were detectable at 1 DPI and then persistently rose during the subsequent five weeks. These results suggest that both variant and classical GPVs can infect Pekin ducklings. The present work contributes to the understanding of pathogenicity of GPV to Pekin ducks and may provide clues to pathogenesis of GPV-related SBDS.

1. Introduction

Goose parvovirus (GPV) was originally recognized as the causative agent of Derzsy's disease, affecting both goslings (*Anser anser domestica*) and Muscovy ducks (*Cairina moschata*) (Fang, 1962; Derzsy, 1967; Gough, 1991; Glávits et al., 2005). In general, Pekin ducks (*Anas platyrhynchos*) are believed to be refractory to GPV infection (Hoekstra et al., 1973; Gough et al., 1981; Gough, 1991). Since 2014, GPV has been noted to cause short beak and dwarfism syndrome (SBDS) in Pekin ducks (strain Cherry Valley) in China (H. Chen et al., 2015; Li et al., 2016; S. Chen et al., 2016; Yu et al., 2016; Ning et al., 2017), which resembles SBDS occurred in mule ducks (intergenetic cross of Pekin and Muscovy ducks) in France in 1971/1972 and in Poland in 1995 (Villatte, 1989; Palya et al., 2009; Woźniakowski et al., 2012). Phylogenetic analysis of partial VP1 and VP3 sequences revealed that GPV strains from SBDS in mule and Pekin ducks belong to a distinct lineage of GPV, namely West-European lineage (Palya et al., 2009; Ning et al., 2017). Studies undertaken in Hungary have shown that SBDS in mule ducks can be reproduced with a GPV of mule duck-origin following infection at one day and 2 weeks of age, but not with a GPV isolate from Derzsy's disease (Palya et al., 2009). The findings suggest that the GPV isolate from SBDS (designated variant) is distinct from the classical GPV strain from Derzsy's disease in terms of pathogenicity to mule ducks.

It has been shown previously that changes of amino acids at the receptor binding site of the capsid protein can influence host range and pathogenicity of parvoviruses (P. Wu et al., 2000, 2006; Govindasamy et al., 2003; López-Bueno et al., 2006; Z. Wu et al., 2006; Kailasan et al., 2015). Thus, the occurrence of SBDS in Pekin ducklings seems to be attributed to alteration of host ranges of GPV caused by variation of the virus. However, compared with classical GPV strains, the capsid proteins of Chinese variant GPV strains contain only two common substitutions, which are located in positions distantly from the potential receptor binding sites (Ning et al., 2017). Based on the recent description of natural and experimental hosts of GPV (Palya, 2013), the embryo-adapted GPV given by parenteral route can infect young and adult Pekin ducks. Therefore, we consider it likely that the GPV-caused SBDS might not be attributed to host range switch of the virus from goslings to Pekin ducklings (Ning et al., 2017). To search for clues to pathogenesis of the GPV-related SBDS, we investigate the pathogenicity of a variant GPV of Pekin duck-origin (JS1) and a classical GPV of goose-origin (H) and their capacity to replicate in 2-day-old Pekin ducklings.

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http://dx.doi.org/10.1016/j.vetmic.2017.08.020
Received 20 June 2017; Received in revised form 26 August 2017; Accepted 28 August 2017

2. Materials and methods

2.1. Virus strains

The JS1 strain of variant GPV was originally isolated in 2015 in Jiangsu province of China from a 2-week-old Pekin duckling exhibiting signs typical of SBDS (Ning et al., 2017). The H isolate of classical GPV was kindly provided by Mr. J. Wang, ZhongshengTiaozhan Bioengineering, Tianjin, China. The virus was originally isolated in 2002 from Derzsy' disease of goslings. For the purpose of this paper, the viruses were passaged three times in embryonated goose eggs. Clarified suspensions of allantoic fluids and embryo bodies harvested from dead goose embryos between 3 and 5 days after inoculation were prepared as described previously (Wang et al., 2013).

2.2. Animal experiments

All procedures involving animals were approved by the Animal Welfare and Ethical Censor Committee at China Agricultural University and Beijing Administration Committee of Laboratory Animals (Approval ID SYXK [Jing] 2015–0028).

Ninety newly hatched Pekin ducklings were derived from a commercial hatchery located in Beijing, where SBDS had never been observed. At day 2, the ducklings were divided into three groups (30 birds/group), and reared in different isolators. The challenged groups were inoculated intramuscularly with the JS1 and H viruses at the dose of 5×10^6 ELD_{50} per bird respectively, and the control group with 0.5 ml of phosphate buffered saline (PBS). The birds were monitored daily for 35 days.

Body weight as well as width and length of beak of birds were measured weekly. At 6 and 12 h (H) as well as 1, 3, 7, 14, 21, and 28 days post infection (DPI), three ducks were selected randomly from each group and blood was collected. Subsequently, the selected ducks were euthanized, and tissues (e.g., heart, liver, and spleen) and ileac contents were sampled. At 35 DPI, the length and width of the tongues of all remaining ducks were measured, and samples were collected from three ducks as above. Ducks in the three groups were examined using X-rays. All samples were subjected to detection of viral load. The tissues were also submitted to histopathological examination. Serum samples were prepared from blood samples for detection of antibody against GPV.

2.3. Histopathological examination

The organs were fixed in 4% neutral formalin at room temperature for 48 h, embedded in paraffin, and cut into 5-μm-thick sections. After deparaffinization, the sections were stained with haematoxylin and eosin (H & E). Pathological changes were observed under an Olympus microscope (Olympus, Tokyo, Japan).

2.4. DNA extraction

Tissues and ileac contents collected from ducks were processed as 20% suspensions in PBS. DNA was extracted from 200 μl of each suspension prepared from the duck samples and the GPV isolates using a DNA Isolation Kit for Cells and Tissues (Aidlab, Beijing, China) according to the manufacturer's instructions. For blood samples, DNA was extracted using a DNA Isolation Kit for Blood (Aidlab, Beijing, China) according to the manufacturer's instructions. Each DNA was eluted in 100 μl elution buffer.

2.5. GPV quantification

GPV loads in duck samples were quantified by using a GPV quantitative real-time PCR (qPCR) assay. Briefly, MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/) was used to align genomic sequences of GPVs, including the JS1 (Ning et al., 2017), QH15 (Yu et al., 2016), and SDLC01 (H. Chen et al., 2015) isolates of variant GPV and the B (Zádori et al., 1995), YZ99-6 (Wang et al., 2014), 82-0321, 82–0321 V, 06-0329 (Shien et al., 2008), and VG32/1 (Tatár-kis et al., 2004) isolates of classical GPV. Two primer pairs were designed over conserved regions of GPVs, using Primer Premier 5.0 (Premier Biosoft International). The primer pair, 477f (5′-CTTCCGGTTTAGTTCATTCG-3′) and 4673r (5′-GCCAGGAAGTGCTTTATTTG-3′), encompassed the full-length NS and VP1-coding regions of GPVs and was employed to amplify a PCR product of 4197 base pair (bp) from DNA of the JS1 isolate. The PCR product was then cloned into the pGEM-T Easy Vector (Promega, Madison, USA), resulting in a recombinant plasmid pGEM-JS1. The primer pair, 3010f (5′-CCGAACCTGTGGCAGCATCT-3′) and 3186r (5′-TGTTGTAGCTTGGCAG GACC-3′), was designed to amplify a 177 bp fragment from the VP3-coding region of the GPV genome. The specificity of primer pair 3010f and 3186r was evaluated by conventional PCR using pGEM-JS1 and DNAs extracted from the JS1 and H isolates as templates, followed by nucleotide sequence determination and analysis of the amplified fragments (Ning et al., 2007). The reactions and conditions for conventional PCR were the same as previously reported (Chang et al., 2000).

The concentration of pGEM-JS1 was measured by Biodropsis BD-1000 ultraviolet spectrophotometry (Beijing Oriental Science and Technology Development, Beijing, China). 10-fold serial dilutions (10^{-3}–10^{-10}; corresponding to 2.42×10^7–2.42 copies/μl) of the vector construct pGEM-JS1 were employed to generate standard curve for the qPCR assay. The reaction mixture (20 μl) contained 2 μl of template, 0.4 μl of each of forward primer 3010f and reverse primer 3186r (10 μM), and 10 μl of AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China). qPCR was conducted using the following conditions: initial annealing at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, and 60 °C for 30 s.

The specificity of the qPCR assay was tested in amplification reactions containing DNA/cDNA samples of several frequently occurring pathogens in waterfowl, including GPV (isolates JS1 and H), duck enteritis virus (DEV), avian influenza virus (AIV), Newcastle disease virus (NDV), duck hepatitis A virus 1 (DHAV-1), DHAV-3, duck astrovirus 1 (DAstV-1), duck reovirus (DRV), and Tembusu virus (TMUV). The sensitivity for GPV detection was measured by using serial 10-fold dilutions (10^{-1}–10^{-11}; corresponding to 2.42×10^9–2.42×10^{-1} copies/μl) of pGEM-JS1 as templates.

For determination of viral load, DNAs extracted from bloods, tissue samples and ileac contents were subjected to qPCR performed as described above. Each DNA sample was tested for three times.

2.6. GPV antibody detection

Antibodies in sera were analysed using a GPV VP3-based enzyme-linked immunosorbent assay (ELISA) as described previously (Zhang et al., 2010). The sera were inactivated at 56 °C for 30 min, diluted 1:100 using 5% skimmed milk, and then used in the assay. The OD values were measured at 450 nm using an ELISA microplate reader. Each sample was tested for three times. A serum sample collected from an infected duck was considered as positive if its OD450 value was more than twice the value obtained from the uninfected control group.

2.7. Statistical analysis

The data was calculated as mean ± standard deviation (SD). For comparison of groups, we used the GraphPad Prism software version 6.01 (GraphPad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered statistically significant.