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Short communication

The newly emerging duck-origin goose parvovirus in China exhibits a wide range of pathogenicity to main domesticated waterfowl

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ABSTRACT

Short beak and dwarfism syndrome virus (SBDSV) is a newly emerging distinct duck-origin goose parvovirus that belongs to the genus *Dependovirus*. Our previous studies have found that SBDSV was highly pathogenic to Cherry Valley ducklings and mule ducklings. However, little is known about its pathogenicity to other waterfowls. In the present study, the pathogenicity of SBDSV was evaluated in domesticated waterfowl including Muscovy ducklings, Sheldrake ducklings and domestic goslings. All experimentally infected birds exhibited remarkable growth retardation, anorexia and diarrhea similar to naturally infected birds. Interestingly, atrophic beaks and protruded tongues were not observed in all infection groups. At necropsies, no diagnostic pathological lesions were observed. Viral antigens existed in most organ tissues such as heart, liver, spleen, kidney, pancreas and intestine. All ducks in Muscovy duckling and Sheldrake duckling infected groups and 70% goslings in infected groups were seropositive for goose parvovirus (GPV) antibodies at 21 dpi with the average titers as $2^{8.4}$, $2^{6.9}$, $2^{4.0}$, respectively. Muscovy ducklings were more prominent in viral load and weight loss with a higher GPV antibodies titer than Sheldrake ducklings and goslings. Taken together, SBDSV exhibits a wide range of pathogenicity to main domesticated waterfowl with variable symptoms and cause considerable economic losses in China.

1. Introduction

Short beak and dwarfism syndrome virus (SBDSV) is a distinct duck-origin goose parvovirus (GPV) and has been emerging as a severe health threat to duck flocks with notable growth retardation and beak atrophy since 2015 in China (Chen et al., 2015a, 2016c, 2015b; Li et al., 2016; Wang et al., 2016; Yu et al., 2016). The outbreak of short beak and dwarfism syndrome (SBDS) disease is very quick and wide in different duck-producing areas of China in recent years, including Fujian, Shandong, Anhui, Shanghai, Hebei, Zhejiang and Guangdong provinces (Chen et al., 2016a, 2016c; Li et al., 2016; Yu et al., 2016). It was easy to find clinical cases in mule duck and Cherry valley duck flocks. The typical symptoms are notably atrophic beak, protruded tongue, remarkable growth retardation, fractured legs and diarrhea. So far, no obvious diagnostic pathological lesion has been observed in sick or dead ducklings. The morbidity varied from 10% to 100% and mortality is low as 0–10% due to disease resistance with age (Chen et al., 2016b, 2016c, 2015b). Basing on the significant stunted growth, this disease has caused a great economic loss in China.

In our previous study, a causative agent of SBDS had been isolated from mule ducklings and identified as a distinct GPV variant strain (SBDSV M15) (Chen et al., 2016c). SBDSV M15 is most closely related to European and Chinese SBDS GPV strains (Wang et al., 2016). In order to confirm SBDSV's pathogenicity in other domesticated waterfowl, 3-day-old Muscovy ducklings, Sheldrake ducklings and domestic goslings which were free from GPV antibodies were infected with a high dose of SBDSV M15. The disease incidence, beak dimensions, body weights, viral antigens distributions and serum antibody titers were monitored at indicated time points post infection.

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2. Materials and methods

2.1. Antibodies and reagents

GPV MAb E16 and GPV MAb-based latex agglutination reagent for detection of GPV antigen were prepared in our lab. FITC (fluorescein isothiocyanate) goat anti-mouse IgG was purchased from BOSTER (Wuhan, China).

2.2. Birds, virus strain and infection

SBDSV M15 strain was isolated from mule ducks with SBDS disease in Fujian, China and cultured in 11-day-old specific-pathogen-free (SPF) embryonated duck eggs (Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences). Three-day-old healthy Muscovy ducklings, Sheldrake ducklings and goslings were checked waterfowl parvovirus antibodies using latex agglutination inhibition assay (LAI) described previously (Chen et al., 2016c; Zhu et al., 2012b). Thirty Muscovy ducklings, Sheldrake ducklings and goslings, respectively, which were free from waterfowl-parvovirus antibodies were marked individually, then equally and randomly divided into six groups. Three groups were used for infection and the other groups served as control. Each bird in infection groups was inoculated orally with 0.8 mL SBDSV M15 ($2^{6.0}$ LA titer, fourth-passage allantoic fluid virus). Each bird in control groups was infected with 0.8 mL sterile phosphate buffered saline (PBS). All birds were monitored daily for clinical signs such as growth retardation, short beak and feather disorder. Body weights and beak dimensions were measured at 14, 21 and 28 days post infection (dpi), respectively.

2.3. Detection of viral antigen by indirect immunofluorescence assay

Five birds of each group were euthanized at 10 dpi by intravenous pentobarbital sodium. Tissue samples including liver, spleen, heart, intestine, kidney and pancrea were collected for detection of GPV antigen using indirect immunofluorescence assay (IFA) described previously (Chen et al., 2016c; Zhu et al., 2012a).

2.4. Quantification of viral load in different tissues by real-time PCR assay

The specific primers targeting SBDSV VP3 were designed as following: forward, 5′-GAATGGAGTAGGGTGGAA-3′, reverse, 5′-GCCATCAGTCTTCGGTAT-3′, probe, FAM-5′-GGAATGGTGTGGCA-GAAC-3′-TAMRA. The tissue samples at 10 dpi including heart, liver, spleen, pancreas, kidney and intestine were collected and homogenized in DMEM (40%w/v), and the viral loads of homogenates were determined by real-time PCR. Viral DNA was extraction by using OMEGA Viral DNA kit (Lot. D3892-01). Premix Ex Taq (Probe qPCR mix), primer and probe were bought from TaKaRa. The real-time PCR reaction volume was 20 μL, containing 0.4 μL 0.2 μmol/L forward primer, 0.4 μL 0.2 μmol/L reverse primer, 0.8 μL 0.4 μmol/L probe, 10 μL Premix Ex Taq, 0.2 μL ROX Refernce Dye, 6.2 μL ddH_2O and 2.0 μL DNA template. The cycling conditions were 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 30 s.

2.5. Serological detection by latex agglutination inhibition assay

Serum samples were collected at 21 dpi for detection of GPV specific antibodies by using latex agglutination inhibition (LAI) assay as described previously (Chen et al., 2016c).

2.6. Statistical analysis

Data were represented as the mean ± SD. Statistical significance was determined by Student's *t*-test analysis. Differences were considered statistically significant with $P < 0.05$.

2.7. Ethics statement

All experimental procedures involving birds were performed in accordance with the regulations of the Administration of Affairs Concerning Experimental Animals, approved by the State Council of China.

3. Results

3.1. SBDSV infection remarkably inhibited weight gain in experimentally infected birds

Birds in control groups were healthy during whole periods of the experiment. Birds in infection groups got sick after 7 dpi and showed symptoms of listlessness, flocking together, anorexia and water-like diarrhea (Fig. 1). No birds died in infection groups. There was no significant difference in body weight gain between infection group and control group during the first week after inoculation. However, the infection groups exhibited remarkable growth retardation after 14 dpi. The body weights of SBDSV infected birds were much lower than those of the control groups at 14, 21, 28 dpi, respectively ($P < 0.05$). SBDSV infection strongly inhibited Muscovy ducklings 41%, 43%, 57.5% of weight gain at 14, 21, 28 dpi, respectively. The rates of weight loss in Sheldrake duckling infection group were 5.9%, 23.6%, 27.9% at 14, 21, 28 dpi, respectively, as comparing to control group. Consistently, SBDSV infection also remarkably inhibited goslings 19.0%, 32.0%, 36.0% of weight gain at 14, 21, 28 dpi, respectively. No diagnostic pathological lesions were observed in all infected birds. Although the average beak lengths and widths of infection groups were shorter than those of control groups, no notable atrophic beaks and protruded tongues were observed in all infection groups (Table 1).

3.2. Viral antigens were detected in most organ tissues

Since a monoclonal antibody (E16) specific for GPV was developed in our previous work, we developed an indirect immunofluorescence assay (IFA) for detecting GPV antigen. Viral antigen was detected in most tissues of infection group birds, including intestine, heart, liver, spleen, kidney and pancreas. No viral antigen was detected in any of sampled tissue from the control birds (Fig. 2).

Subsequently, viral load in different tissues of birds at 10 dpi were detected by using real-time PCR. The correlation coefficient value R^2 was 0.999, which could be used for quantifying the viral copy number (Fig. 3A-B). In all infection groups, viral DNA copy number in liver and pancreas were higher than in heart, spleen, kidney and intestine at 10 dpi, and Muscovy ducks contain higher viral load than Sheldrake ducks and goose (Fig. 3C). No positive viral DNA was detected in the control birds.

3.3. High level GPV antibodies detected in SBDSV infected birds

All birds were exsanguinated at 21 dpi for detection of special antibodies of GPV using LAI test described previously. Muscovy duck and Sheldrake duck infection groups developed high level GPV antibodies with the average titers of $2^{8.4}$ and $2^{6.9}$, respectively. However, only 70% birds in gosling infection group were seropositive for GPV antibodies with a low average titer of $2^{4.0}$. The antibody titers in duck infection groups were significantly