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LETTER TO THE EDITOR

Isolation and characterization of a goose parvovirus from Yan goose

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Goose parvovirus (GPV) infection, also known as Derzsy's disease, is an acute or sub-acute septic infectious disease in goslings and Muscovy ducklings. It mainly occurs in goslings younger than 1 month, and all goose breeds including white goose, gray goose and Muscovy duck are susceptible to the virus (*1–3*). Generally, digestive tract inflammation, characterized by sausage-shaped embolisms in small intestine, is the main gross lesion at necropsy in GPV-infected goslings and Muscovy ducklings (*4*).

Yan goose, originating in Lu'an region, Anhui Province, China, is one of rare medium-sized gray goose breeds in China. With its increasing number in recent years, GPV infection has occasionally occurred in this breed in China. Our team first isolated GPV Yan-1 strain from a Yan gosling in 2011 and has uploaded its *VP* gene sequence to the GenBank (Acc. No. KR265070). A GPV infection case in Yan geese was also reported in 2014 (*5*). However, to our knowledge, no works have been conducted to elaborate the features of Yan goose-origin GPV isolates, and the whole genome sequence of Yan goose-origin GPV is also not available in the GenBank. We isolated a GPV strain named Yan-2 from a non-immunized dead Yan gosling and the phylogenetic analysis based on its full-genome sequence demonstrated its close genetic relationship with the Y strain from Muscovy duck and SHFX1201 from swan. Immunohistochemical assay also revealed its tissue tropism against liver, intestine and nerve.

In 2013, Yan goslings, which were reared in a farm in Anhui Province of China and not immunized with any GPV vaccines, showed clinical symptoms, including serous secretions from nostrils, feed refusal, generous drinking, instability of gait, and leg paralysis. The diseased geese died within 5–7 days post the onset. The necropsy revealed ecchymotic liver, congestive brain, kidney swelling, and characteristic sausage-shaped embolisms in duodenum and ileum. Duodenum, jejunum, liver, brain, spleen, lung, kidney, pancreas, and bursa of Fabricius were collected from the dead Yan Gosling, fixed in 4% (v/v) paraformaldehyde, and prepared into paraffin sections. The HE staining of these paraffin sections demonstrated hyperemia and inflammatory cell infiltration in the liver; villus necrosis and abscission as well as exfoliation of intestine epithelium mixed with inflammatory cells and cellulose-like exudates in the duodenum, ileum and jejunum; capillary injection and telangiectasia in the brain; and cloudy swelling of renal tubular epithelial cells.

After observation of gross lesions, virus fluid was obtained from the liver of a 20-day-old dead Yan gosling using routine method. The virus fluid was inoculated into 12-day-old non-immunized goose embryos via allantoic cavity, 0.2 ml per embryo, followed by incubation at 37°C. The results showed that most of the inoculated goose embryos died between 60 and 120 hr post the inoculation, while the control goose embryos survived normally. The inoculated goose embryos manifested severe systemic hemorrhage, mostly in heart, liver and kidney. The allantoic fluid was harvested from the dead embryos except those, which died within 24 hr,

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Abbreviations: GPV = goose parvovirus

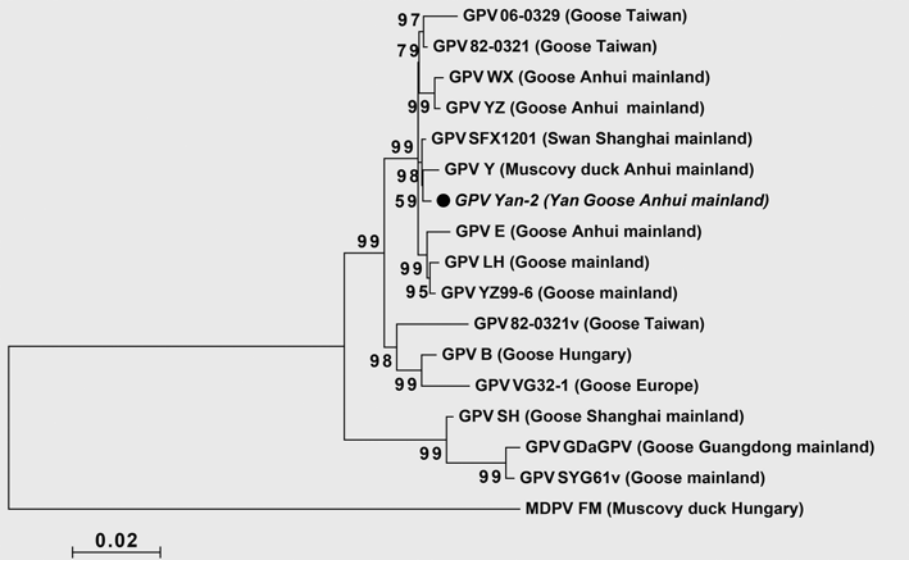

Fig. 1

Full-genome sequence-based phylogenetic analysis of GPV Yan-2 strain

An unrooted phylogenetic tree was generated by the distance-based neighbor-joining method using MEGA 6.0 software. The reliability of the tree was assessed by bootstrap analysis with 1,000 replications.

and preserved at 4°C. The agar diffusion test showed the virus suspension reacted with GPV antibody-positive serum other than Newcastle disease virus or avian influenza virus antibody-positive serum (All sera were prepared and preserved by Animal Pathology Laboratory of College of Animal Science and Technology, Anhui Agricultural University, Hefei, China), revealing the presence of GPV. The hemagglutination test showed the allantoic fluid harvested from the dead goose embryos could not coagulate duck, chick, rabbit and mouse erythrocytes, which is consistent with the feature of parvovirus. The transmission electron microscope observation of the viral fluid demonstrated the virus particles were spherical, non-enveloped, 20–25 nm in diameter, with typical characteristics of parvovirus. Therefore, the age of onset, clinical symptoms, pathological changes and laboratory detection confirmed the diagnosis of the GPV infection in the Yan gosling, and the isolate was thus named GPV Yan-2.

To observe the expression of viral antigen in different tissues, the immunohistochemical analysis was performed using mouse anti-GPV monoclonal antibodies (presented by Key Laboratory of Jiangsu Preventive Veterinary Medicine, Yangzhou, China) as primary antibodies, goat anti-mouse IgG as secondary antibodies, streptavidin-biotin complex (Boster) as enzymes, and DAB reagent as substrates. The results revealed the GPV Yan strain was widely distributed in the cytoplasm and nuclei of some intestinal epithelial cells in the duodenum, ileum and jejunum, hepatocytes, nerve cells in the brain, and renal tubular epithelial cells, while no positive signal was detected in other tissues. These results are also supported by other researches (*6, 7),* demonstrating Yan geese are also the natural reservoir of GPV.

The full-genome sequence was obtained using the same primers and methods given in the references (*8*). The full genome sequence of GPV Yan-2 strain was 5,106 bp in size (GenBank Acc. No. KR136258), consisting of inverted terminal repeat, 1,844-bp NS and 2,199-bp VP, showing structural features of GPV genome.

Genomic homology was analyzed between the isolate and all GPVs with whole-genome sequences available in the GenBank, using the neighbor-joining method based on 1, 000 replicates in Mega 6.0 software. The GPV Yan-2 strain shares a high degree of nucleotide homology with other GPV strains (94.1%–99.7%). The phylogenetic analysis revealed the GPV Yan-2 strain has a close genetic relationship with Muscovy duck-origin GPV Y strain and swan-origin GPV SHFX1201 strain (*9*), respectively, isolated in Anhui Province and adjacent Shanghai City, indicating the regional prevalence of the GPV Yan-2 strain. However, it has a farer genetic relationship with the Chinese GPV vaccine strains SYG61v (*10*) and GDaGPV than other strains isolated in China mainland, suggesting it might be a new epidemic strain. Moreover, it is located in the phylogenetic tree far from the nodes of the sequences for the standard B strain and vaccine strain VG32-1 (*11, 12*) (Fig. 1).

Therefore, the isolation of GPV Yan-2 from the Yan goosling suggests Yan goose may serve as a natural reservoir

of GPV, and liver, intestine and nerve are its target tissues for Yan goose. Further studies are needed to describe its other features and pathological mechanisms.

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