

# Novel goose-origin astrovirus infection in geese: the effect of age at infection

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**ABSTRACT** Since 2017, a serious infectious disease characterized by visceral gout has emerged in China's main goose-producing regions. The disease has caused huge economic losses to China's goose industry. In our previous study, we determined that the pathogen causing gout in goslings is a novel goose-origin astrovirus, designated as AStV/SDPY/Goose/1116/17 (AStV-SDPY) strain. To investigate the effect of host age on the outcome of novel goose-origin astrovirus infection, 200 1-day-old healthy goslings were selected to be experimentally infected at 1, 5, 15, 25, and 35 D of age. It was shown in experimental infection that the AStV-SDPY strain was highly pathogenic in goslings aged 1 to 15 D, causing growth repression, severe visceral urate deposition, and even death, whereas goslings infected at 25 and 35 D of

age showed mild symptoms. Histopathologic examination indicated that lesions occurred mainly in the kidney and liver of infected goslings, which is correlated to the severity of clinical signs and gross lesions. Viral RNA was detected in all representative tissues, and virus shedding was detected continuously within 15 D after inoculation. Higher viral copy number, especially in vital organs such as the liver and kidney, was developed in the goslings infected at 1 to 15 D of age than older geese. In addition, clinical chemistry and inflammatory cytokines showed that younger geese are more sensitive to AStV infection. Overall, our study demonstrates that the pathogenicity of AStV-SDPY in goslings is partly associated with the age of infection, laying a foundation for further study of the pathogenic mechanism of this virus.

**Key words:** novel goose-origin astrovirus, goose, viral load, histopathology, age

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## INTRODUCTION

Astrovirus was named under the suggestion of Madeley and Cosgrove who described the virus as a star-shaped morphology, 28 nm in diameter, and found in feces of infants with diarrhea under electron microscopy (Madeley and Cosgrove, 1975; Kurtz et al., 1979). The family Astroviridae was classified as 2 genera, namely, *Mamastrovirus* (mammal astrovirus, MAstV) and *Avastrovirus* (avian astrovirus, AAstV) as per the different types of infected hosts (Pantin-Jackwood et al., 2011). Mammal astrovirus genus is further divided into human,

cat, swine, sheep, and mink astroviruses (Rivera et al., 2010). On the other hand, classified as a part of the genus astrovirus, AAstV are known to cause infection in poultry, thus causing economic losses to farms (Koci and Schultz-Cherry, 2002). Avian astroviruses have been associated with avian diseases, including enteritis in turkeys, chickens, and guinea fowl, mild growth depression in chickens, mild nephritis in chickens, and hepatitis in ducklings (Chu et al., 2012). Currently, at least 6 genetically distinct AAstV, including the avian nephritis virus, chicken astrovirus, turkey astrovirus (TAstV) type 1, TAstV type 2, duck hepatitis virus type 2, and duck hepatitis virus type 3, have been identified based on the species of origin and viral genome characteristics (Errol et al., 2008). The molecular characterization of the different types of avian astroviruses shows great genetic variability. Coinfection with different AAstV strains have been reported, and this can result in recombination between different strains (Strain et al., 2008; Pantin-Jackwood et al., 2011). The

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diversity and ecology of AAstV in other wild avian species have not been fully explored, and such information will facilitate our understanding of the origins, evolution, and epidemiology of AAstV in poultry.

As nonenveloped viruses, AAstV are characterized by a positive-sense, single-stranded RNA viruses similar to all other members of the Astroviridae family. Ranging from 6,100 to 7,900 nt in length, the genomes of these viruses are arranged in 3 open reading frames (ORF) (ORF1a, ORF1b, and ORF2), as well as a short 5'-untranslated region, a 3'-untranslated region, and a poly-A tail (Kapoor et al., 2009; Wan et al., 2018). Open reading frame 1a and ORF 1b encode nonstructural proteins including transmembrane, serine proteases, coiled-coil, viral genome-linked protein, nuclear localization signal, and RNA-dependent RNA polymerases. All astroviruses have conserved slippery sequence in the overlap region between ORF 1a and ORF1b (AAAAAAC), which directs the synthesis of ORF 1 ab fusion protein (Lewis and Matsui, 1996). Open reading frame 2 encodes capsid precursor protein, which is important for inducing the host immune response and has the greatest diversity in the genome (Arias and DuBois, 2017).

Since November 2016, an infectious disease characterized by gout, hemorrhage, and kidney swelling has affected goslings in the major goose-producing regions of Jiangsu, Shandong, Anhui, Guangdong, Henan, Liaoning, Hunan, Fujian, and Zhejiang provinces of China. The economic losses caused by this outbreak are estimated to be between 1.2 and 1.5 billion yuan. The mortality of the disease was up to 50%, resulting in severe economic losses in China's goose industry (Yang et al., 2018). We first determined that the pathogen of this disease was a novel goose-origin astrovirus (Niu et al., 2018). The phylogenetic analysis revealed that SDPY strain was in the independent small-branch-divergent strain group but belongs to the large branch of AAstV group 1. The results have indicated that the SDPY isolate might be a novel strain of AAstV (Niu et al., 2018; Yuan et al., 2019). In this study, AStV-SDPY isolate was used to intranasally infect geese of 5 different ages, and a systematic investigation was conducted, including detection of viral loads, BW measurement, histopathology, and clinical chemistry, to investigate the effect of host age on the outcome of novel goose AStV infection.

## MATERIALS AND METHODS

### Ethics Statement

All animal infection experiments were conducted in accordance with the Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China) and were approved by the supervision of the Animal Protection and Utilization Committee of Shandong Agricultural University (approval No. SDAUA-2018-51).

## Virus

A novel goose-origin astrovirus strain named AStV-SDPY (GenBank Accession No. MH052598.1) was characterized by gout in goslings (Yang et al., 2018) and used as the challenge virus in experimental infection. AStV-positive tissues were homogenized with physiological saline and filtered through a 0.22- $\mu$ m filter. The filtrate was inoculated into allantoic cavities of 10-day-old goose embryos under aseptic conditions. The embryos were then incubated at 37 °C, and embryos that died within the first 24 h were discarded. Allantoic fluid was harvested at 96 h after inoculation under aseptic conditions, and the virus was passaged 4 times (Wei et al., 2019). The challenge virus was strictly tested for sterility and the presence of goose extraneous agents by PCR assay (including the avian influenza virus, Newcastle disease virus, avian orthoreovirus, fowl adenovirus, goose parvovirus.) The challenge virus has an infectivity titer of  $10^{-5.25}$  EID<sub>50</sub>/0.2 mL, which was calculated using the Reed and Muench assay (Reed and Muench, 1938). PCR was performed using the LongGene Gradient Thermal Cycler (LongGene, Hangzhou, China) in a total volume of 40  $\mu$ L. All primers were synthesized by TaKaRa (Dalian, China).

## Animals

One-day-old healthy goslings were purchased from the geese hatchery in Liaocheng city, Shandong province, China. Before experiments, serum samples and cloacal swabs were collected from goslings and tested by quantitative PCR to confirm that they were free of AStV infection or other goose pathogens (including the avian influenza virus, Newcastle disease virus, avian orthoreovirus, fowl adenovirus, goose parvovirus.)

## Animal Experiments

A total of 200 goslings were randomized into 10 different groups (including 5 experimental groups and 5 control groups). Goslings in the experimental groups were infected with 0.5 mL of AStV-SDPY strain at 1 D of age (S1 group), 5 D of age (S2 group), 15 D of age (S3 group), 25 D of age (S4 group), and 35 D of age (S5 group) by intranasal route. Goslings in the control groups were inoculated with the same volume of sterile PBS (pH 7.2–7.4) in the same way.

**Table 1.** Mortality of goslings after infection with the AStV-SDPY strain.

Group	Inoculation age		Mortality after SDPY infection (dpi)					Total
			5	6	7	8	9	
S1	1	Dead geese	-	-	1	1	1	3
S2	5	Dead geese	1	-	1	-	-	2
S3	15	Dead goose	-	-	1	-	-	1
S4	25	Dead goose	-	1	-	-	-	1

Abbreviation: dpi, days postinfection.

All goslings were maintained in specific pathogen-free isolators of Shandong Agricultural University Research Containment Facility with ad libitum feeding until inoculation. The infected groups and control groups were reared in separate biosafety level 2 rooms at 23°C. Each group was individually housed in stainless steel cages. All goslings were provided normal access to water and commercial maintenance food mash (New Hope LIUHE, Beijing, China) (CP, crude fiber, calcium, phosphorus, and vitamins) twice a day. Feeding and management of all goslings were performed in accordance with the established humane procedures and biosecurity guidelines with living conditions of 40–60% relative humidity and a 12L:12D cycle. All goslings were monitored and recorded for 15 D after infection.

### Sample Collection

At 3, 6, 9, 12, and 15 D postinfection (dpi), 3 goslings (did not die after infection) were randomly selected from each group to collect blood, and then, goslings were euthanized with intravenous pentobarbital sodium (New Asia Pharmaceutical, Shanghai, China) and necropsied, along with the collection of their tissues (including the heart, liver, spleen, lung, kidney, bursa, thymus, pancreas, brain, and proventriculus). At 1, 3, 5, 7, 9, 11, 13, and 15 dpi, 3 goslings were randomly selected from each group to measure their weight and collect cloacal swab samples. Tissues and cloacal swab samples were assayed for viral loads by quantitative real-time PCR, and the blood was

centrifuged, with serum samples being separated and stored at –20 °C. The biochemical indexes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and uric acid (UA), were investigated by Taian Animal Central Hospital. Cytokines were detected using the double-antibody sandwich ELISA kit of goose IL-6 and interferon gamma (ML Bio, Shanghai, China) as per the manufacturer's directions.

### Detection of Viral Loads

Viral RNA of tissues and swab samples were extracted by TRIzol Reagent (CWBIO, Beijing, China). Quantitative real-time PCR was performed using the TaKaRa One-Step Prime Script RT-PCR Kit (TaKaRa, Dalian, China) in a total volume of 20 µL based on the Roche LightCycler 96 Real-Time PCR System (Roche, Basel City, Switzerland). The program of quantitative real-time PCR was performed by using the TaqMan one-step RT-PCR method established in our laboratory (Yin et al., 2020). All primers and probe were synthesized by TaKaRa (Dalian, China).

### Histopathology

The histopathology analysis of infected goslings was conducted. After 72 h of fixation in 10% neutral formalin buffer, 5 mm sections of tissue (the heart, liver, spleen, lung, kidney, bursa, thymus, pancreas, brain, proventriculus) were routinely processed and embedded in

**Table 2.** Gross lesions in goslings of 5 different age groups on different day after inoculation.

Group	dpi <sup>1</sup>	Gross lesions				
		Heart	Liver	Kidney	Joint	Proventriculus
S1	3	0/3 <sup>2,-3</sup>	0/3,-	2/3,+	0/3,-	0/3,-
	6	2/3,++	1/3,++	2/3,++	0/3,-	1/3,+++
	7	1/1,+++	1/1,+++	1/1,+++	1/1,+++	1/1,+++
	8	1/1,++	1/1,++	1/1,+++	1/1,+++	1/1,++
	9	1/4,++	2/4,++	2/4,++	1/4,++	1/4,+
	12	0/3,-	1/3,+	1/3,++	0/3,-	0/3,-
S2	3	0/3,-	0/3,-	2/3,+	0/3,-	0/3,-
	5	1/1,++	1/1,++	1/1,+++	1/1,+++	1/1,++
	6	0/3,-	1/3,+	1/3,++	0/3,-	1/3,+
	7	1/1,+	1/1,++	1/1,+++	1/1,++	1/1,+
	9	0/3,-	2/3,+	1/3,+	0/3,-	0/3,-
	12	0/3,-	1/3,+	1/3,+	0/3,-	0/3,-
S3	3	0/3,-	0/3,-	1/3,+	0/3,-	0/3,-
	6	0/3,-	0/3,-	1/3,++	0/3,-	0/3,-
	7	1/1,+	1/1,++	1/1,++	1/1,++	0/1,-
	9	0/3,-	0/3,-	1/3,+	0/3,-	0/3,-
S4	12	0/3,-	0/3,-	1/3,+	0/3,-	0/3,-
	3	0/3,-	0/3,-	0/3,-	0/3,-	0/3,-
	6	0/3,-	1/3,+	1/3,+	0/3,-	0/3,-
	9	0/3,-	0/3,-	1/3,+	0/3,-	0/3,-
S5	12	0/3,-	0/3,-	0/3,-	0/3,-	0/3,-
	3	0/3,-	0/3,-	0/3,-	0/3,-	0/3,-
	6	0/3,-	0/3,-	0/3,-	0/3,-	0/3,-
	9	0/3,-	0/3,-	1/3,+	0/3,-	0/3,-
	12	0/3,-	0/3,-	0/3,-	0/3,-	0/3,-

Abbreviation: dpi, days postinfection.

<sup>1</sup>Geese were routinely necropsied at 3, 6, 9, 12, and 15 dpi; geese necropsied in between were found dead.

<sup>2</sup>Number of geese with lesions/Geese examined.

<sup>3</sup>Severity of gross lesions: -, no lesions; +, mild; ++, moderate; +++, severe.

paraffin, which were then stained with hematoxylin and eosin and examined with an optical microscope (Eclipse E100; Nikon, Japan).

### Statistical Analysis

Statistical comparisons were analyzed by two-tailed Student unpaired *t* test using Graph Prism (GraphPad Software Inc.). Data were expressed as means  $\pm$  SD. Data from the 5 infection groups were compared with those from the control groups in the same chart. Statistical significance was set at  $P < 0.05$ .

## RESULTS

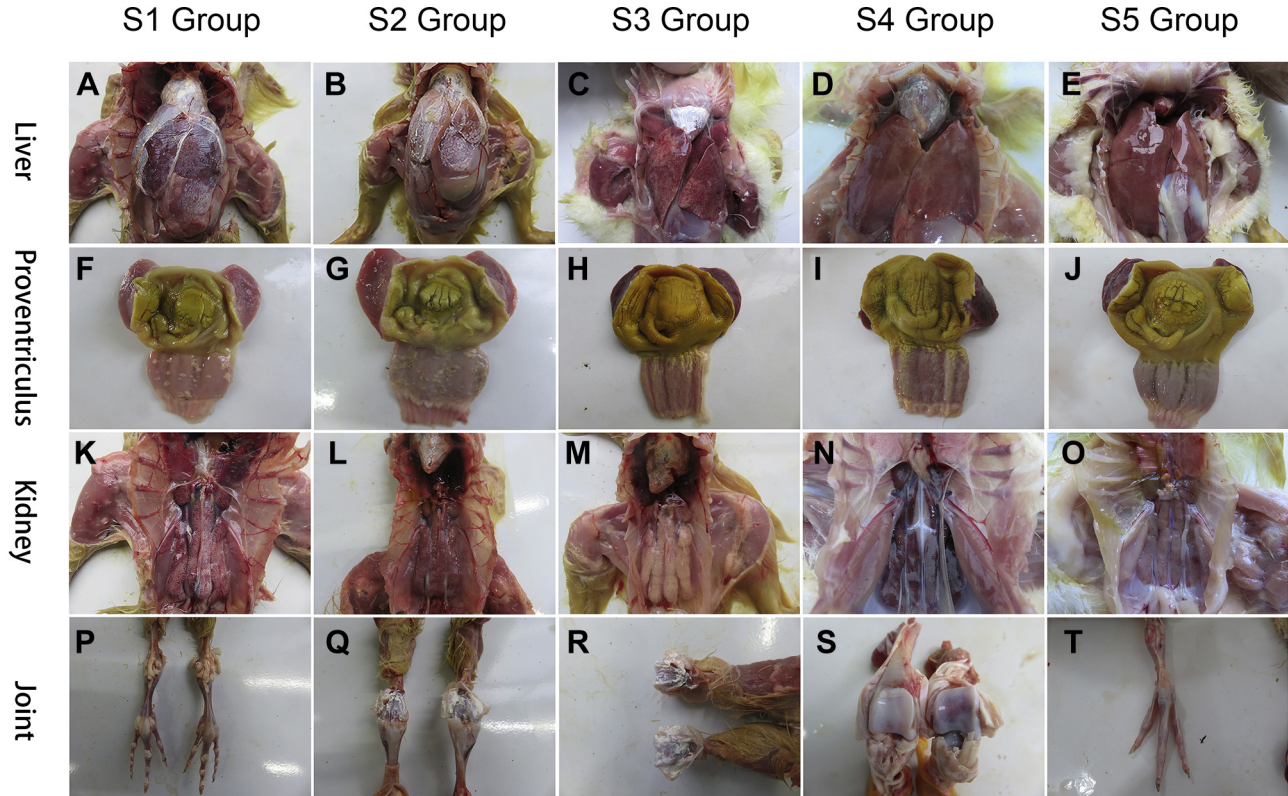
### Clinical Signs and Gross Lesions

To investigate the effect of age on the infection of AStV-SDPY in goslings, we monitored their clinical signs and gross lesions for 15 D after inoculation. In S1, S2, and S3 groups, depression and inappetence were observed at 3 to 5 dpi. One goose died at 7, 8, and 9 dpi in group S1; one died at the fifth and seventh dpi in S2 group; one died at seventh dpi in group S3; one died at sixth dpi in group S4; whereas geese in S5 group did not die (Table 1). Gross lesions in goslings infected at different days of age are summarized in Table 2. Severe

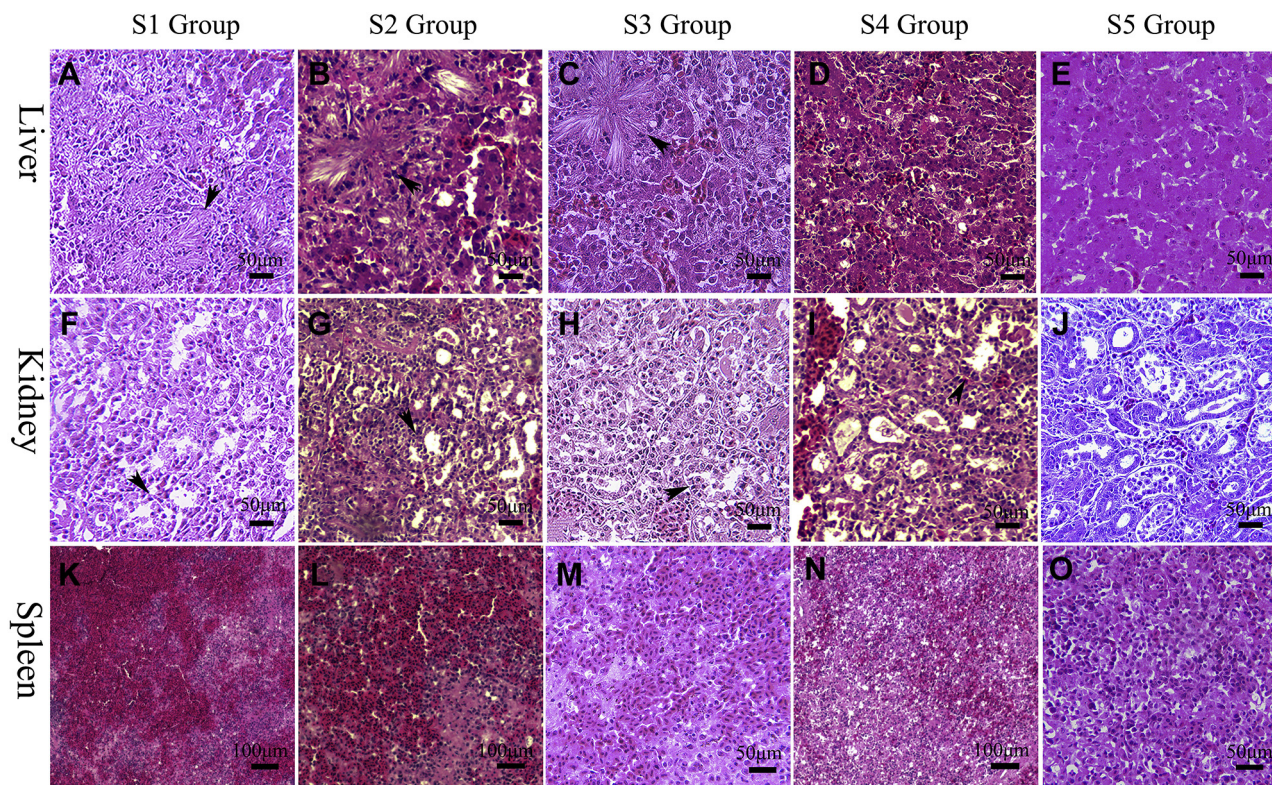
lesions were found in S1 and S2 groups at 6–9 dpi (Table 2), whose gross lesions included white urate on the surface of the organs, such as the liver, heart, kidney, proventriculus and joint; kidney, and liver enlargement (Figures 1A, 1F, 1K, 1P, 1B, 1G, 1L, 1Q). Similar but milder lesions were observed in the S3 group infected with AStV-SDPY (Figures 1C, 1H, 1M, 1R), whereas no obvious lesions were found in 25- and 35-day-old goslings infected with AStV (Figures 1D, 1E, 1N, 1O, 1S, 1T). No mortality or any clinical symptoms were found in control goslings.

### Microscopic Changes of Organs

Organs collected from infected goslings in S1 group showed significant microscopic changes, with the lesions appearing in well-vascularized organs. In S1 group, urate crystals were found in liver tissue (Figure 2A), diffuse hemorrhage in the spleen (Figure 2K), and more severe renal lesions, including renal tubular epithelial necrosis and shedding (Figure 2F). In S2 and S3 groups, urate crystals were also observed in the hepatocyte (Figures 2B, 2C), hemorrhage was found in spleen tissue (Figures 2M, 2L), along with glomerular swelling and renal tubular shedding in the kidney (Figures 2G, 2H). Milder lesions in kidney of geese



**Figure 1.** Gross lesions of goslings infected with AStV-SDPY at 5 different ages. (A–C) Urate deposits on the surface of the liver and heart in groups S1, S2 and S3, whereas there were no obvious urate in the S4 (D) and S5 (E) groups; urate deposits on the surface of the proventriculus in groups S1 (F) and S2 (G), no urate deposition were observed in S3 (H), S4 (I), and S5 (J) groups; Severe hemorrhage and swellings appeared in the kidneys of geese in groups S1 (K), S2 (L), mild swellings of kidneys in group S3 (M), no obvious lesions were observed in groups S4 (N) and S5 (O); white urate appeared in joints of goslings in groups S1 (P), S2 (Q), and S3 (R), whereas the joints in the S4 (S) and S5 (T) groups had smooth mucosa and no urate deposits.



**Figure 2.** Pathologic changes of the ASTv-SDPY infected goslings at 5 different ages. (A–C) Urate deposits (black arrow) on the liver (H&E) observed in groups S1, S2 and S3. (D, E) Liver (H&E) in groups S4 and S5, no urate deposits. (F, G) Kidney (H&E) in groups S1 and S2, renal tubular epithelial cell severe exfoliation (black arrow). (H) Renal tubular epithelial cell exfoliation (black arrow) in group S3. (I, J) H&E-stained kidney in groups S4 and S5, renal tubular epithelial cells intact. (K) H&E-stained spleen, S1 group, severe hemorrhage of splenocyte. (L) Spleen (H&E), S2 group, diffuse hemorrhage of splenocyte. (M) Spleen (H&E), S3 group, mild bleeding. (N, O) Spleen (H&E), S4 and S5 groups, goslings infected at 25 and 35 d of age, no microscopic lesions were observed. Abbreviation: H&E, hematoxylin and eosin.

infected at 25 and 35 D of age (Figures 2I, 2J) and no urate crystals were observed in the hepatocyte (Figures 2D, 2E). No microscopic lesions were observed in the control goslings.

### Viral Loads in Tissues

Viral copies detected from 10 organs in geese infected at different day of age are shown in Figure 3. Viral RNA tended to be more frequently detected in tissues of the younger geese. Infection resulted in detectable virus in all tissues examined of the 5 age groups inoculated with ASTv-SDPY at 3 dpi, peaked on 6 dpi, and declined on 9 to 15 dpi. Among all organs, the kidney had the highest viral loads, followed by the liver and spleen. At 3, 6, 9, and 12 dpi, the viral copies in the kidney of 1-day-old infected goslings were higher ( $P < 0.05$ ) than those of the geese infected at 25 and 35 D of age (Figures 3A–3D), indicating there was a strong correlation between the viral copies and the host age. No viral RNA was detected in the control groups.

### Cloacal Viral Shedding

Viral copies could be first detected in cloacal swabs as early as 1 dpi (Figure 3F). The loads of shedding virus within the first few dpi are proportional to the time,

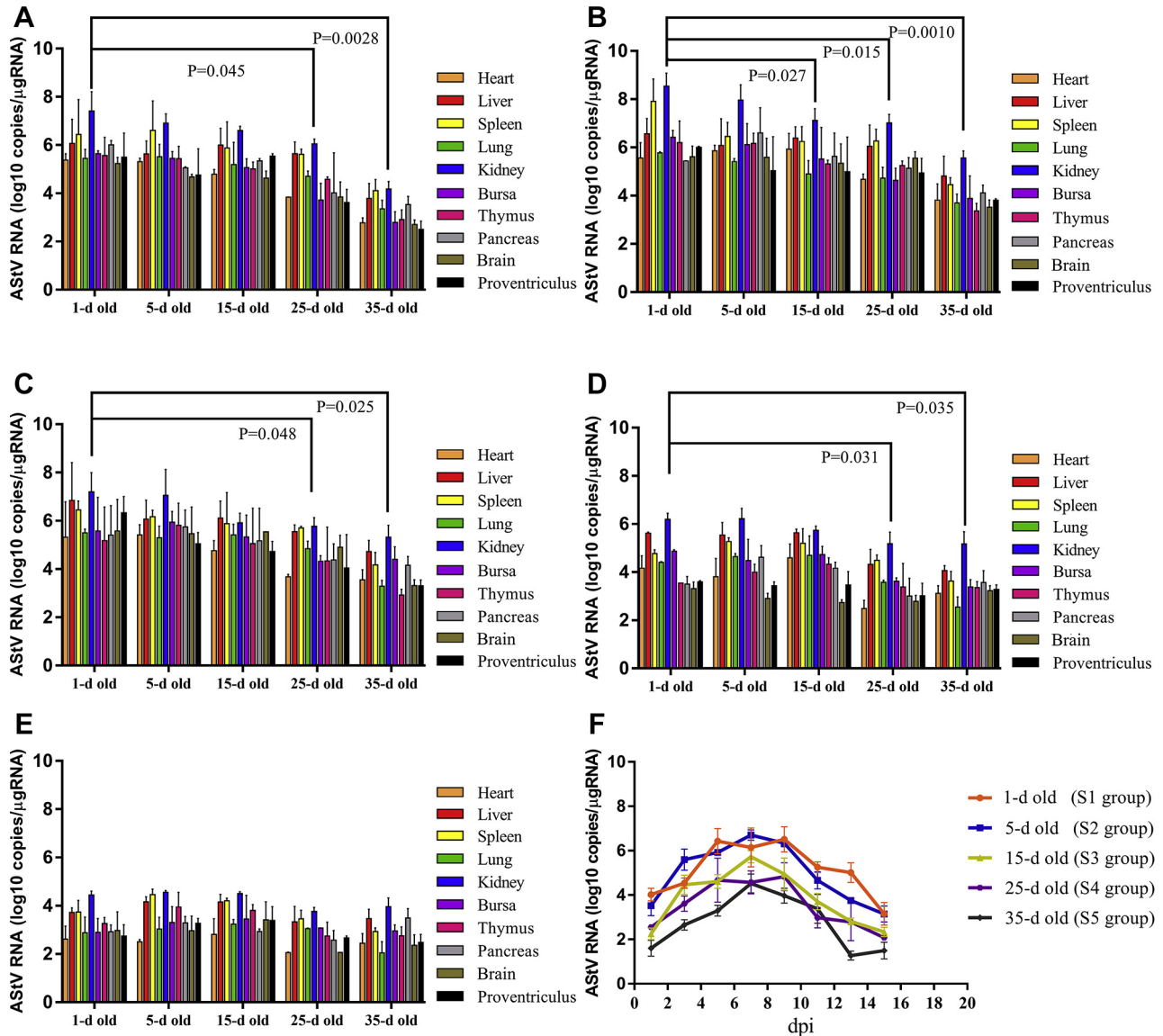
reached a peak value at 5 to 7 dpi, followed by the decline of viral load. The viral copies of each infected group were different at the same time. More specifically, the viral copies detected from cloacal swabs in goslings infected at 1 (S1), 5 (S2), and 15 (S3) D of age were higher than that in goslings infected at older days of age (Figure 3F). No positive viral RNA was recorded in the control goslings in this study.

### ASTv-SDPY Infection Inhibits BW Gain

To explore the effect of SDPY infection on the BW of goslings, the weight changes of the infected and control geese were examined. The results demonstrated that infection inhibited ( $P < 0.01$ ) the weight gain of geese aged 1–15 D (Figures 4A–4C). The effect of SDPY on the BW of the S4 (Figure 4D) and S5 (Figure 4E) groups was not obvious.

### Clinical Chemistry

Organ damage of the goslings was investigated through clinical chemistry, and the results showed that the serum enzyme activities of the infected geese increased. As indicators for evaluating liver damage, the activity of ALT and AST in serum significantly increased in S1, S2, and S3 groups (Figure 5). The



**Figure 3.** Normalized ASTV copy numbers (log<sub>10</sub>) per 1 µg total RNA in the internal organs of experimentally infected goslings collected at 3 (A), 6 (B), 9 (C), 12 (D), and 15 (E) dpi. Statistically significant differences ( $P < 0.05$ ) of viral loads in kidney between 1-day-old infected group and the other 4 age groups. (F) Cloacal virus shedding after the goslings in S1–S5 groups were infected with the ASTV-SDPY. Note: Each sample was analyzed in triplicate. Error bars were expressed as SD of the means ( $n = 3$ ). Abbreviation: dpi, days postinfection.

activity of ALT in S1 and S2 groups was higher ( $P < 0.01$ ) at 3, 6, 9, and 12 dpi (Figures 5A, 5D), and the AST level in S1 and S2 groups was also higher ( $P < 0.01$ ) than that in control groups at 3–9 dpi (Figures 5B, 5E). The levels of ALT and AST in the serum of infected goslings at 15 D of age (S3 group) were lower than those infected at 1 and 5 D of age (S1 and S2 groups) but still higher than that in control goslings at 3 to 6 dpi (Figures 5G, 5H). Uric acid activity was used to evaluate renal damage, finding that UA activity in all infected groups was higher ( $P < 0.01$  in groups S1–S4 and  $P < 0.05$  in group S5) than that in the control groups, and higher UA level was detected in geese serum of the younger groups (Figures 5C, 5F, 5I, 5L, 5O).

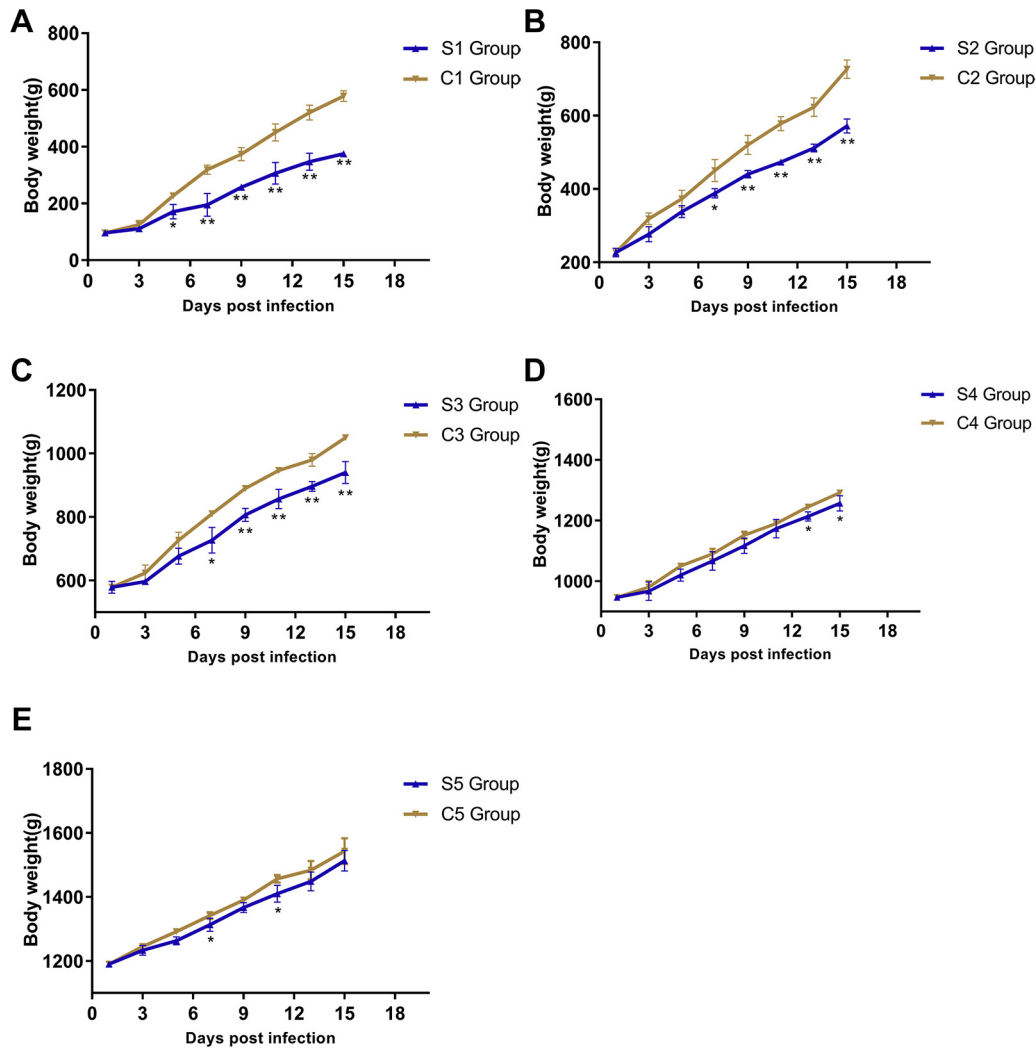
### Changes of Cytokines

As Figure 6 shows, the levels of IL-6 and interferon gamma in each infected group were higher than that in the control groups after infection with SDPY, suggesting

that stronger immune response was observed after infection with SDPY. The levels of IL-6 and interferon gamma in 1-day-old and 5-day-old goslings peaked at 3 dpi, whereas the older geese tended to reach the peak at 6 to 9 dpi.

## DISCUSSION

The sudden outbreak and rapid spread of serious infectious disease which can mainly cause visceral urate deposition in goslings has caused huge economic losses to the Chinese goose industry. A novel goose astrovirus was eventually confirmed as the causative agent of this disease in goslings (Yang et al., 2018). The phylogenetic analysis revealed that the isolate belongs to a novel strain of AAstV. Avian astroviruses have been recognized to cause problems in poultry and have been proved to be responsible for diarrhea, growth depression,



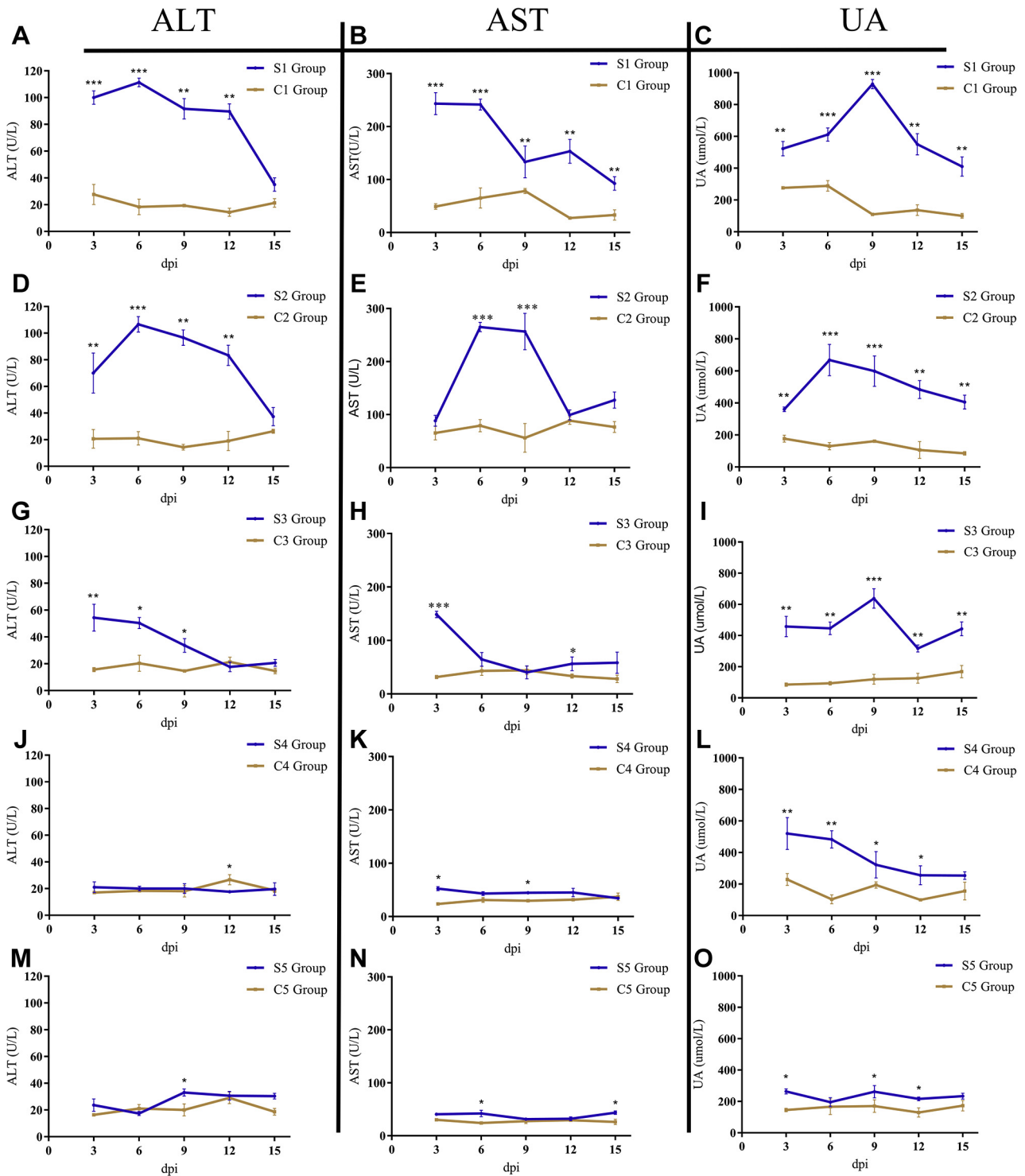
**Figure 4.** Weight changes of goslings after infected with AStV-SDPY. (A) 1-day-old (S1) group (1–15 dpi); (B) 5-day-old (S2) group (1–15 dpi); (C) 15-day-old (S3) group (1–15 dpi); (D) 25-day-old (S4) group (1–15 dpi); (E) 35-day-old (S5) group (1–15 dpi). Note: All values are presented as the mean BW  $\pm$  SD. Asterisks indicate statistically significant differences compared with the control group. \* $P < 0.05$ ; \*\* $P < 0.01$ . Abbreviation: dpi, days postinfection.

nephritis, and enteritis (Reynolds et al., 1987). Chicken astroviruses have been reported to induce gout disease in chickens (Bulbule et al., 2013), and the susceptibility of turkeys to TAsVs at different ages has been reported (Awe et al., 2015). At present, a few studies have reported the genomic characteristics of novel goose astrovirus causing fatal gout in goslings (Liu et al., 2018; Chen et al., 2020), with some reports about the pathogenicity of astrovirus (Zhang et al., 2018). To the best of our knowledge, there are few reports that age at infection affected the outcome of AStV infection in goslings.

The present study has focused on the necropsy lesions, microscopic changes, viral loads, enzymes, and cytokines in serum and weight changes to study the outcome of SDPY infection in goslings of 5 different ages. Experimental infection could reproduce the clinical signs and gross lesions associated with the gout disease. The incidence of infected geese reached 100%, but the mortality rate was relatively low, 15% in the 1-day-old infected group, 10% in the 5-day-old infected group, and 5% in the 15- and 25-day-old infected groups. The high

morbidity and low mortality might be related with the infection route and dose and the host age. Meanwhile, older geese are more likely to survive AStV-SDPY infection than younger geese and changes in susceptibility to AStV were observed as goslings mature. AStV infection caused damage to the immune organs, leading to multi-organ dysfunction and inducing replication of other pathogenic microorganisms, which was responsible for high mortality rate of natural outbreaks (Yu et al., 2019). The difference of lesions in goslings infected at 25–35 D of age was obvious, especially in liver and kidney where the gross and microscopic lesions were milder than goslings infected at 1–15 D of age. The immune organs of the goslings, with advancing age, gradually mature, thus inhibiting the replication of AStV in vivo (Ti et al., 2015).

Infection can reduce feed intake and change the efficiency of feed conversion ratio, thus leading to growth suppression and serious economic losses (Moser and Schultz-Cherry, 2005). AStV infection at earlier age could cause an adverse effect on BW gain, suggesting



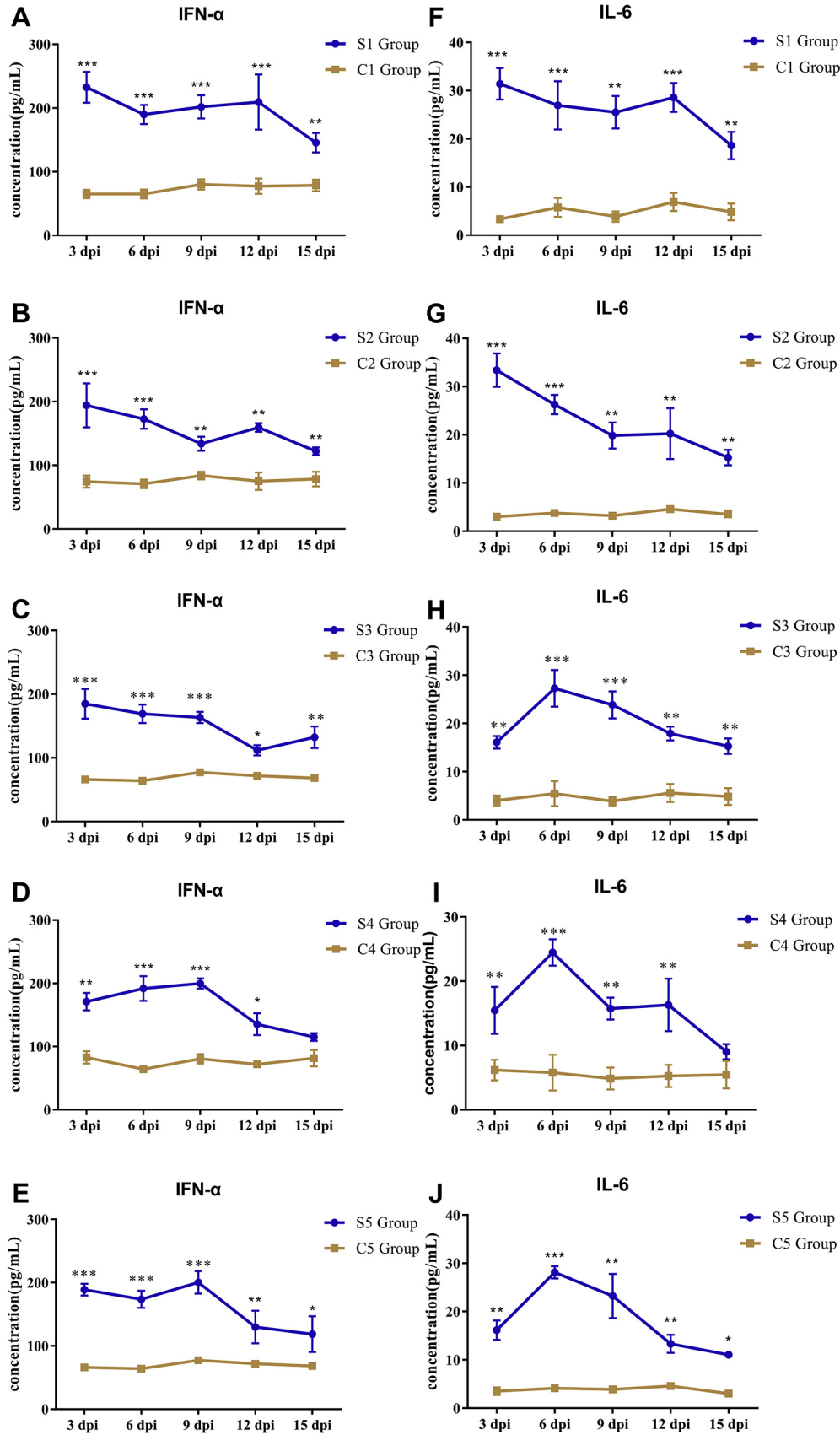
**Figure 5.** Dynamics of ALT, AST, and UA in serum of the geese after artificially beinginfected with AStV-SDPY. (A, D, G, J, M) Levels of ALT in infected groups (S1–S5) and control groups (C1–C5) (3–15 dpi). (B, E, H, K, N) Levels of AST in infected groups (S1–S5) and control groups (C1–C5) (3–15 dpi). (C, F, I, L, O) Levels of UA in infected groups (S1–S5) and control groups (C1–C5) (3–15 dpi). Note: Data are expressed as the mean  $\pm$  SD. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; dpi, days postinfection; UA, uric acid.

that we should strictly prevent the spread of AStV in the newborn goose flock. Microscopic lesions were typical and severe in the liver, kidney, and spleen of 1-day-old, 5-day-old, and 15-day-old goslings, respectively. Twenty-five-day-old and 35-day-old infected goslings showed mild microscopic lesions. The microscopic lesions were correlating with the changes of clinical signs, gross

lesions, and BWloss. It was indicated that the kidney, liver, and spleen were the target organs of AStV infection in geese.

In the early stages of infection, viral RNA was detected in 10 organs including the brain. The presence of AStV in different vital organs suggests that AStV have broader tropism (Padmanabhan and Hause,





**Figure 6.** Dynamics of IL-6 and interferon alpha in serum of the geese after being artificially infected with ASTv-SDPY. (A–E) Levels of IL-6 in groups S1–S5 compared with C1–C5 groups (3–15 dpi); (F–J) levels of interferon alpha in groups S1–S5 compared with C1–C5 groups (3–15 dpi). Note: Data are expressed as the mean ± SD. Asterisks indicate statistically significant differences compared with the control group. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . Abbreviations: dpi, days postinfection; IFN, interferon.

2016). It should be noted that the tissue tropism of AStV implies that the virus may cause greater harm to goslings. Clinically, a rational and standardized immunization program can be established to prevent the spread of the disease. The viral loads of 10 organs peaked at 6 dpi, more specifically, AStV first entered the blood circulation and reached the peak with blood entered the tissues. The viral loads examined in the kidney were higher than that in other organs. In addition, the age gradient of goslings infected with SDPY was designed, and there were significant differences in viral copies detected among goslings infected at different days of age. Statistically, viral RNA was more frequently detected in infected goslings aged between 1 and 15 D, and significantly lower viral copies were detected in tissues of infected goslings aged between 25 and 35 D, demonstrating that difference of clearance against AStV was developed as goslings grew over time.

AStV RNA was continuously detected in cloacal swabs of infected goslings, similar to the trend of organ viral loads. The efficiency of viral replication in host appears to be associated with induction of clinical signs and gross and microscopic lesions especially in vital organs such as the liver and kidney. Different copies of viral RNA detected in cloacal swabs may indicate that the difference in host defenses for fighting disease in goslings at different day of age. Viral nucleic acids released from the cloaca contaminate feed and drinking water, causing more widespread infections, indicating that it is urgent to take effective measures to control the spread of disease (Liu et al., 2016).

Serum biochemical indexes analysis serves as a useful research or diagnostic tool (Andreasen et al., 1996). Serum chemistry analytes were used to study the effects of AStV-SDPY infection on the liver and kidney of geese in the present study. Aspartate aminotransferase run into the blood when hepatic cells are destroyed, which will increase their enzyme levels rapidly (Fan et al., 2014). As the cells are repaired, its content gradually decreases. One percent hepatocyte necrosis is responsible for a 1-fold increase in serum ALT (Kew, 2000; Kim et al., 2008), necrosis of massive hepatocyte after AStV-SDPY infection lead to ALT levels increased rapidly. Approximately 70% of the UA is excreted through the kidneys and 30% through the gastrointestinal tract (Tian et al., 2012). AStV infection damaged the kidney of infected geese, the rate of kidney urate formation is greater than the excretory capacity of the urinary organs, and then caused gout (Tang, 2017). AStV replicated in goslings caused severe lesions to the liver and kidney, which mainly help explain why the levels of ALT, AST, and UA in serum of infected geese are higher than those in the control geese. Organ damage caused disorders in the enzyme system, leading to the release of enzymes from cells into blood. The levels of enzymes in the serum of goslings increased after infection, but the change in the serum enzymes of infected goslings aged 1 to 15 D was more obvious. The changes of serum enzymes were consistent with gross lesions.

IL-6 was produced by a variety of lymphocytes and nonlymphocytes, in the liver; IL-6 is an important inducer of the acute phase response and infection defense (Schmidt-Arras and Rose-John, 2016). Interferon alpha belonging to the type I interferon family is important in the host response to infection, interferon gamma is a potent antiviral agent inhibiting viral replication and conferring cellular resistance to viral infection (Campbell et al., 1999). In this study, IL-6 and interferon alpha increased after infection, and the body was in an emergency immune state. IL-6 and interferon alpha in the serum inhibited virus replication, which caused the viral loads decreased after reaching a maximum. In addition, IL-6 and interferon alpha levels in younger geese peaked earlier after infection, suggesting that younger geese are more sensitive to AStV-SDPY infection than older geese.

Age-related resistance to AStV infection has been observed in turkeys with increased susceptibility of younger turkeys to TAsTV (Awe et al., 2015). In our study, age-related differences in the resistance to AStV-SDPY infection were observed with younger goslings being more susceptible. However, the pathogenesis of this effect is needed to be elucidated. The authors think the novel goose astrovirus vaccine should be developed as soon as possible to protect young susceptible geese. In summary, it is emphasized in this study that age factor should be considered when taking preventive measures.

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## REFERENCES

- Andreasen, Jr, J.R., C. B. Andreasen, A. B. Sonn, and D. C. Robeson. 1996. The effects of haemolysis on serum chemistry measurements in poultry. *Avian Pathol.* 25:519–536.
- Arias, C. F., and R. DuBois. 2017. The astrovirus capsid: a review. *Viruses* 9:15.
- Awe, O. O., K.-i. Kang, M. Ibrahim, A. Ali, M. Elaiash, Y. M. Saif, and C.-W. Lee. 2015. Age-related susceptibility of turkeys to enteric viruses. *Avian Dis.* 59:207–212.
- Bulbule, N. R., K. D. Mandakhalikar, S. S. Kapgate, V. V. Deshmukh, K. A. Schat, and M. M. Chawak. 2013. Role of chicken astrovirus as a causative agent of gout in commercial broilers in India. *Avian Pathol.* 42:464–473.
- Campbell, I. L., T. Krucker, S. Steffensen, Y. Akwa, H. C. Powell, T. Lane, D. J. Carr, L. H. Gold, S. J. Henriksen, and G. R. Siggins. 1999. Structural and functional neuropathology in transgenic mice with CNS expression of IFN- $\alpha$ 1. *Brain Res.* 835:46–61.
- Chen, Q., X. Xu, Z. Yu, C. Sui, K. Zuo, G. Zhi, J. Ji, L. Yao, Y. Kan, Y. Bi, and Q. Xie. 2020. Characterization and genomic analysis of emerging astroviruses causing fatal gout in goslings. *Transbound. Emerg. Dis.* 67:865–876.

- Chu, D. K. W., C. Y. H. Leung, H. K. K. Perera, E. M. Ng, M. Gilbert, P. H. Joyner, A. Grioni, G. Ades, Y. Guan, J. S. M. Peiris, and L. L. M. Poon. 2012. A novel group of avian astroviruses in wild Aquatic Birds. *J. Virol.* 86:13772–13778.
- Errol, S., L. A. Kelley, S. C. Stacey, S. V. Muse, and M. D. Koci. 2008. Genomic analysis of closely related astroviruses. *J. Virol.* 82:5099–5103.
- Fan, S., J. Zheng, Z. Duan, N. Yang, and G. Xu. 2014. The influences of SE infection on layers' production performance, egg quality and blood biochemical indicators. *J. Anim. Sci. Biotechnol.* 5:4.
- Kapoor, A., L. Li, J. Victoria, B. Oderinde, C. Mason, P. Pandey, S. Zaidi, and E. Delwart. 2009. Multiple novel astrovirus species in human stool. *J. Gen. Virol.* 90:2965–2972.
- Kew, M. C. 2000. Serum aminotransferase concentration as evidence of hepatocellular damage. *Lancet* 355:591–592.
- Kim, W. R., S. L. Flamm, A. M. Di Bisceglie, and H. C. Bodenheimer. 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology* 47:1363–1370.
- Koci, M. D., and S. Schultz-Cherry. 2002. Avian astroviruses. *Avian Pathol.* 31:213–227.
- Kurtz, J., T. Lee, J. Craig, and S. E. Reed. 1979. Astrovirus infection in volunteers. *J. Med. Virol.* 3:221–230.
- Lewis, T. L., and S. M. Matsui. 1996. Astrovirus ribosomal frame-shifting in an infection-transfection transient expression system. *J. Virol.* 70:2869–2875.
- Liu, N., M. Jiang, M. Wang, F. Wang, B. Zhang, and D. Zhang. 2016. Isolation and detection of duck astrovirus CPH: implications for epidemiology and pathogenicity. *Avian Pathol.* 45:221–227.
- Liu, N., M. Jiang, Y. Dong, X. Wang, and D. Zhang. 2018. Genetic characterization of a novel group of avastroviruses in geese. *Transbound. Emerg. Dis.* 65:927–932.
- Madeley, C., and B. Cosgrove. 1975. Viruses in infantile gastroenteritis. *Lancet* 306:124.
- Moser, L. A., and S. Schultz-Cherry. 2005. Pathogenesis of astrovirus infection. *Viral Immunol.* 18:4–10.
- Niu, X., J. Tian, J. Yang, X. Jiang, H. Wang, H. Chen, T. Yi, and Y. Diao. 2018. Novel goose astrovirus associated gout in gosling, China. *Vet. Microbiol.* 220:53–56.
- Padmanabhan, A., and B. M. Hause. 2016. Detection and characterization of a novel genotype of porcine astrovirus 4 from nasal swabs from pigs with acute respiratory disease. *Arch. Virol.* 161:2575–2579.
- Pantin-Jackwood, M. J., K. O. Strother, E. Mundt, L. Zsak, J. M. Day, and E. Spackman. 2011. Molecular characterization of avian astroviruses. *Arch. Virol.* 156:235–244.
- Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Epidemiol.* 27:493–497.
- Reynolds, D., Y. Saif, and K. Theil. 1987. A survey of enteric viruses of turkey poults. *Avian Dis.* 31:89–98.
- Rivera, R., H. H. Nollens, S. Venn-Watson, F. M. D. Gulland, and J. F. X. Wellehan. 2010. Characterization of phylogenetically diverse astroviruses of marine mammals. *J. Gen. Virol.* 91:166–173.
- Schmidt-Arras, D., and S. Rose-John. 2016. IL-6 pathway in the liver: from physiopathology to therapy. *J. Hepatol.* 64:1403–1415.
- Strain, E., L. A. Kelley, S. Schultz-Cherry, S. V. Muse, and M. D. Koci. 2008. Genomic analysis of closely related astroviruses. *J. Virol.* 82:5099–5103.
- Tang, S. C. W. 2017. Gout: a disease of Kings. *Contrib. Nephrol.* 192:77–81.
- Ti, J., L. Zhang, Z. Li, D. Zhao, Y. Zhang, F. Li, and Y. Diao. 2015. Effect of age and inoculation route on the infection of duck Tembusu virus in Goslings. *Vet. Microbiol.* 181:190–197.
- Tian, Y., Y. Chen, B. Deng, G. Liu, Z.-G. Ji, Q.-Z. Zhao, Y.-Z. Zhen, Y.-Q. Gao, L. Tian, and L. Wang. 2012. Serum uric acid as an index of impaired renal function in congestive heart failure. *J. Geriatr. Cardiol.* 9:137.
- Wan, C.-H., C.-T. Chen, L.-F. Cheng, R.-C. Liu, S.-H. Shi, G.-H. Fu, Q.-L. Fu, H.-M. Chen, and Y. Huang. 2018. A novel group of avian Avastrovirus in domestic geese, China. *J. Vet. Med. Sci.* 80:798–801.
- Wei, Z., H. Liu, Y. Diao, X. Li, S. Zhang, B. Gao, Y. Tang, J. Hu, and Y. Diao. 2019. Pathogenicity of fowl adenovirus (FAdV) serotype 4 strain SDJN in Taizhou geese. *Avian Pathol.* 48:477–485.
- Yang, J., J. Tian, Y. Tang, and Y. Diao. 2018. Isolation and genomic characterization of gosling gout caused by a novel goose astrovirus. *Transbound. Emerg. Dis.* 65:1689–1696.
- Yin, D., J. Yang, J. Tian, D. He, Y. Tang, and Y. Diao. 2020. Establishment and application of a TaqMan-based one-step real-time RT-PCR for the detection of novel goose-origin astrovirus. *J. Virol. Methods* 275, 113757.
- Yu, G., Y. Lin, Y. Dou, Y. Tang, and Y. Diao. 2019. Prevalence of fowl adenovirus serotype 4 and Co-infection by immunosuppressive viruses in fowl with hydropericardium hepatitis syndrome in Shandong province, China. *Viruses* 11:517.
- Yuan, X., K. Meng, Y. Zhang, Z. Yu, W. Ai, and Y. Wang. 2019. Genome analysis of newly emerging goose-origin nephrotic astrovirus in China reveals it belongs to a novel genetically distinct astrovirus. *Infect. Genet. Evol.* 67:1–6.
- Zhang, X., D. Ren, T. Li, H. Zhou, X. Liu, X. Wang, H. Lu, W. Gao, Y. Wang, X. Zou, H. Sun, and J. Ye. 2018. An emerging novel goose astrovirus associated with gosling gout disease, China. *Emerg. Microbes Infect.* 7:1–8.