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The effect of Tembusu virus infection in different week-old Cherry Valley breeding ducks

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ABSTRACT

To study the effect of Tembusu virus (TMUV) infection on Cherry Valley Breeding ducks of different ages, 350 five-week-old ducks were divided into 14 groups. Ducks in seven experimental group were respectively infected with 1.265×10^5 mean embryo lethal dose (ELD_{50}) of TMUV-AHQY strain (in 4.2 mL) by intravenous route. Ducks in control groups were inoculated with Phosphate-buffered Saline (PBS) in the same way. Clinical symptoms, gross and microscopic lesions, viral loads and serum antibodies were detected and recorded for 20 days after infection. Some ducks infected at 7 and 21 week s of age showed severe clinical symptoms including depression and inappetence, and no obvious clinical symptoms were seen in other week-old infected ducks. Severe gross lesions including hepatomegaly, meningeal congestion, myocardial hemorrhage, intestinal, myocardial and pulmonary edema were observed in ducks infected at 7, 18 and 21 weeks of age. No or mild gross lesions were observed in ducks infected at 14 and 16 weeks of age. The main microscopic lesions including hyperaemia, degeneration and necrosis of different cells and inflammatory cellular infiltration mainly consisting of mononuclear cells or lymphocytes were observed in ducks infected at 7 and 21 week of age. But relatively intact structures and rare lymphocytic infiltration were presented in ducks infected at 14 and 16 weeks of age. Viral antigen was more frequently observed in organ slices collected from 7 week-old infected ducks and few positive staining was found in 14 and 16 week-old infected ducks. Less viral loads in different tissues and swabs were detected by a quantitative real-time PCR assay. The level of viral loads in the tissues of ducks infected at 14 and 16 weeks of age was very lower than that of ducks infected at 7 and 21 weeks of age. Meanwhile, less viral copy numbers were detected in swab samples collected from 14 and 16 week-old infected ducks. Ducks infected at 14-week-old developed significantly higher serum neutralizing antibody titers than those infected at other week of age. These results indicated that the effect of TMUV infection on Cherry Valley ducks is partly related to weeks of age. 7–10 week-old and 18–21 week-old ducks were more susceptible to TMUV infection, but 14–16 week-old ducks were more resistant to this disease.

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1. Introduction

Tembusu virus (TMUV) infection has been emerging on many egg-laying and breeder duck farms in Southeastern China since 2010, which was characterized by acute anorexia, retarded growth, neurological dysfunction and sharp declines in egg production (Su et al., 2011). TMUV is a member of Ntaya virus (NTAV) group within the family Flaviviridae, genus Flavivirus (Cao et al., 2011). The emergence of TMUV infection has significant effect on waterfowl industry and causes serious economic loss in China.

Epidemiological investigation has revealed that the natural infection with TMUV has been reported in many kinds of domestic ducks including Peking duck, Cherry Valley duck, Shaoxing duck, Jinyun duck, Longyan duck and Khaki-Campbell duck (Tang et al., 2013). In the clinical cases, younger waterfowls infected with TMUV were more susceptible to the virus and showed more severe clinical signs, gross lesions and higher mortality than older folcks. Another study reported that TMUV infection not only infected egg-laying ducks but also 3–21 day-old Peking ducklings. The infected ducklings showed neurological symptoms such as ataxia and paralysis (Yun et al., 2012).

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Recent study bears out that the outcome of TMUV infection in Cherry Valley ducklings is partly age-related and younger ducks are more susceptible to this disease (Sun et al., 2014). These phenomena remind us to hypothesize the outcome of TMUV infection in breeding ducks is also related to age at infection. But less information is available about the effect of age on pathogenicity of TMUV in Cherry Valley breeding ducks.

In this study, different week-old ducks were infected with TMUV by intravenous route to investigate the effect of TMUV on Cherry Valley breeding ducks.

2. Materials and methods

2.1. Animals

350 five-week-old Cherry Valley ducks were purchased from a commercial hatchery under controlled sanitary status in Shandong Province. Those ducks were fed ad libitum in different isolators until they were 7-week-old before the start of experiment. Serum and swab samples were collected from ducks before inoculation to confirm that ducks were TMUV-negative by SNT and qRT-PCR (Sun et al., 2014).

2.2. Virus

The AHQY strain (accession number: KJ740748.1) was originally isolated from an egg-laying duck during an outbreak of TMUV infection in Anhui Province, China. This virus was passed four times in allantoic cavities of 9-day-old SPF chicken embryos and used as the challenge virus in our experiment. The challenge virus has an infectivity titer of 10-3.16 ELD50/0.2 mL, which was calculated using Reed and Muench method (Matumoto, 1949).

2.3. Designing of experiment

350 ducks (175 control ducks and 175 infected ducks, respectively)were divided into 14 different groups (seven groups as experimental groups and the other groups as the control). Ducks in seven experimental groups were infected with 1.265 × 105 mean embryo lethal dose (ELD50) of TMUV-AHQY strain (in 4.2 mL) at 7 week-old, 10 week-old, 12 week-old, 14 week-old, 16 week-old, 18 week-old and 21 week-old by intravenous route respectively. Ducks in control groups were inoculated with Phosphate-buffered Saline (PBS) in the same way. Clinical symptoms, gross and microscopic lesions and serum antibodies were detected and recorded for 20 days after infection.

2.4. Samples

Tissues (brain, heart, spleen, liver, kidney and ovary) were collected from infected ducks sequentially euthanized at days 2, 5, 8, 12, 16 and 20 after infection. And the tissues from control groups were collected in the same way. Clotted blood and swab samples were collected from all birds before slaughter. One part of collected tissues was fixed in 10% buffered neutral formalin to carry out pathological and immunohistochemical studies. Another part of collected tissues and serum were stored at −80 °C until using.

2.5. Histopathology and immunohistochemistry

After 72 h of fixation in 10% buffered neutral formalin, tissues were routinely processed and embedded in paraffin. 4 μm sections were stained with hematoxylin and eosin (HE) following standard histopathological protocols. The sections were placed on glass slides and dried in an oven at 60 °C for 6 h. Thereafter, the sections were putting into graded alcohol liquid (100% ~ 75%) after deparaffined in xylene. Then the sections were staining with hematoxylin for 5 min and added a step of hydrochloric acid alcohol differentiation for 10 s. After immerged in eosin alcohol solution for 1 min, the sections were washed by current water, dehydrated using graded ethanol (90% ~ 100%), vitrification by dimethylbenzene and deposited in neutral balsam.

Viral staining was performed with a mouse-derived monoclonal antibody against TMUV protein E (Chen et al., 2014) diluted at 1:100, and incubated overnight at 4 °C. A goat anti-mouse HRP-conjugated polyclonal serum (Beijing CoWin Biotech Co. Ltd., Beijing, China) was used as the secondary antibody at dilution 1:300. Diaminobenzidine was used as the substrate chromagen, and slices were counterstained with hematoxylin. IHC controls were consisted of slices treated with normal mouse serum instead of the primary antibody and slices from the control ducks (Sun et al., 2014).

2.6. RNA extraction and qRT-PCR

For samples preparation, 2 g of tissues was snap-frozen in liquid nitrogen immediately and stored at −80 °C until being used. Total RNA was extracted from different organs and swab samples

Table 1
Gross lesions in ducks from different age groups at 5 dpi.

Tissues and lesions	07-week-old ducks	10-week-old ducks	12-week-0ld ducks	14-week-old ducks	16-week-old ducks	18-week-old ducks	21-week-old ducks
Brain							
congestion	3/4, +++	2/4, ++	1/4, +	0/4, –	0/4, –	2/4, ++	2/4, ++
dropsy	2/4, ++	1/4, +	0/4, –	0/4, –	0/4, –	2/4, ++	2/4, ++
Heart							
hemorrhage	2/4, ++	1/4, +	0/4, –	0/4, –	0/4, –	1/4, +	2/4, ++
Lungs							
edema	3/4, +++	2/4, ++	2/4, ++	1/4, +	1/4, +	2/4, ++	3/4, +++
Liver							
tumefaction	2/4, ++	2/4, ++	1/4, +	1/4, +	0/4, –	2/4, ++	3/4, +++
intestines							
hemorrhage	3/4, +++	2/4, ++	2/4, ++	1/4, +	1/4, +	2/4, ++	3/4, +++

A. The number of ducks displaying gross signs in the sacrificed ducks inoculated with TMUV.
B. The severity of gross signs: – = no lesion; + = mild; ++ = moderate; +++ = severe.

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