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Effect of age and inoculation route on the infection of duck Tembusu virus in Goslings

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ABSTRACT

Duck Tembusu virus (TMUV) is an emerging flavivirus that has caused variable levels of outbreaks in poultry in recent years. In order to study the effect of age and inoculation routes on the TMUV infection, one hundred healthy domestic 5-day-old and 20-day-old goslings were equally divided into five groups and four experimental groups of goslings were infected with the TMUV-SDSG strain by intravenous and intranasal routes, respectively. Severe clinical signs were observed in goslings infected at 5 days of age, including listlessness, growth retardation, severe neurological dysfunction and even death. However, goslings infected at 20 days of age showed mild symptoms and no mortality. The severity of gross lesions gradually reduced as goslings matured. The severe histopathological changes were observed in 5-day-old infected goslings, including cerebral edema, viral encephalitis, myocardial necrosis, hepatic steatosis, spleen lymphoid cell depletion, pancreatic epithelial cell shedding and interstitial hemorrhage. However, 20-day-old infected goslings showed mild histopathological changes. Viral loads in different tissues were detected by the SYBR Green I real-time PCR assay. The level of viral loads in most of tissues 5-day-old infected goslings was higher than that of 20-day-old infected goslings, correlating with the severity of clinical symptoms and lesions in these tissues. 20-day-old infected goslings developed significantly higher serum neutralizing antibody titers than 5-day-old infected goslings. Furthermore, goslings infected with TMUV intravenously demonstrated more severe clinical signs, lesions and higher viral loads in tissues than those of goslings infected with TMUV intranasally. Therefore, age and inoculation routes can affect the pathogenicity of TMUV in geese and younger geese are more susceptible to the virus. Age and inoculation route factors should be considered in study of the pathogenicity, pathogenesis, folumation of prevention and therapy strategies of TMUV infection in geese.

1. Introduction

Tembusu virus (TMUV) was first isolated from mosquitoes in Kuala Lumpur as early as 1955, and clustered in Ntaya virus group under the family Flaviviridae, genus Flavivirus (Bowen et al., 1975; Karabatsos, 1978; Olson et al., 1983). In 2011, duck TMUV was found in China which caused a decline in egg production in laying ducks as well as neurological dysfunction, including paralysis and twisted necks, and death in ducklings (Cao et al., 2011; Su et al., 2011). Almost all species of ducks were susceptible to TMUV, such as Beijing duck, Shaoxing duck, golden duck, Cherry Valley etc. (Yan et al., 2011). Chickens, geese, sparrows and some birds were also naturally infected and showed clinical signs (Li et al., 2013; Tang et al., 2015, 2012). Soon, the infection spread to the major duck-producing regions quickly and caused enormous economic losses in China.

Although the resistance of geese to pathogens is high, TMUV still causes a severe infection in laying geese, breeder geese and goslings. Laying geese infected with TMUV mainly showed the clinical signs and lesions similar to the infected ducks, including anorexia, weight loss, diarrhea, egg production drop, ovary hemorrhage and follicle rupture (Liu et al., 2012). Goslings infected with TMUV mainly showed neurological symptoms including standing instability and movement disorders (Han et al., 2013; Liu et al., 2012). In the cases of natural TMUV infection, younger ducks and geese were more susceptible to the virus and showed more serious symptoms, lesions and higher mortality than the older flocks. The significant effect of age on the pathogenicity of TMUV in ducks was confirmed by a series of experimental data (Sun et al.,

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2014). However, little information is available about the effect of age on the pathogenicity of TMUV in geese.

In this study, we used two different ages of goslings to investigate the effect of age and inoculation routes on the pathogenicity of TMUV in geese.

2. Materials and methods

2.1. Virus

The TMUV strain was first isolated from a laying duck farm with a drop in egg production in Shandong province in 2011. After the infected duck theca was collected, homogenized and filtered, the supernatant was inoculated in 9-day-old SPF (specific pathogen free) duck embryos (Harbin Veterinary Research Institute). Most inoculated embryos were dead in 72–120 h with severe cutaneous hemorrhages and growth stunting. Allantoic fluid was used as the challenge virus in this study after three passages. The viral titer of challenge virus was $10^{3.5}$ ELD_{50}/0.2 mL (Median embryo lethal dose), determined by using Reed and Muench method (Reed and Muench, 1938). The TMUV strain was named TMUV-SDSG.

2.2. Animal experiments

100 healthy goslings (5-day-old and 20-day-old) were divided equally into five groups. All goslings were fed normally in different isolators until inoculation. Cloacal and tracheal swabs and serum samples were collected from all goslings before inoculation to determine that the goslings were TMUV-negative virologically and serologically by real-time PCR and serum neutralization test (SNT). Two groups of goslings were inoculated with 1 mL of the challenge virus intravenously (i.v) and intranasally (i.n), respectively, at 5 days of age. Another two groups were inoculated in the same way at 20 days of age. The control group was inoculated with 1 mL sterile phosphate-buffered saline (PBS). Water and food were provided ad libitum everyday. Feeding and management of all goslings were performed in according to the established humane procedures and biosecurity guidelines.

All goslings were observed and recorded daily over 12 days. At 3, 6, 9 and 12 dpi, three goslings from four infected groups and the control group were euthanized by carbon dioxide, respectively. Serum and tissue samples (heart, liver, spleen, kidney, pancreas and brain) were collected. One part of the collected tissue samples was stored at −80 °C to determine the viral loads and the other was immediately fixed in 10% neutral buffered formalin for histopathology and immunohistochemistry examination. This research was approved by the Animal Ethics Committee of Shandong (permit number: 20127620).

2.3. Histopathology and immunohistochemistry examinations

After 48 h of fixation in 10% neutral buffered formalin, tissues were embedded in paraffin and cut into sections with 4 mm thickness. The sections were stained with hematoxylin and eosin (H&E) for microscopic examination by using standard histopathology protocols. For immunohistochemistry examination, sections were deparaffinized through xylene, successively hydrated with 95%, 80%, 75% and 50% ethanol and finally washed in distilled water. Sections were immersed in 3% hydrogen peroxide solution for 10 min at room temperature to inactivate endogenous peroxidase and heated to expose antigen at 98 ~ 100 °C. Sections were blocked with 5% goat serum albumin in PBS for 1 h at 37°C and incubated with a mouse-derived monoclonal antibody for TMUV protein E (Chen et al., 2014) overnight at 4 °C. After three washes in PBS, sections were incubated with a goat anti-mouse HRP-conjugated polyclonal serum (TransGen, Beijing, China) for 1 h at 37 °C. After washing three times in PBS, diaminobenzidine was used as the substrate chromagen and sections were counterstained with hematoxylin. The sections were coverslipped with gum and observed under the microscope.

Fig. 1. Clinical signs of goslings inoculated with TMUV. (A and B) 5-day-old goslings inoculated intravenously and intranasally exhibited typical neurological symptoms at 6 dpi. (C and D) 20-day-old goslings inoculated intravenously and intranasally exhibited depression, inappetence and diarrhea at 6 dpi.