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Research paper

Dose-dependent pathogenicity of a pseudorabies virus variant in pigs inoculated via intranasal route

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ABSTRACT

Pseudorabies (PR) or Aujeszky's disease (AD), caused by pseudorabies virus (PRV), is an economically important viral disease in many countries. The modified live vaccine Bartha-K61 strain has played an important role in the control of PR in many countries including China. Since late 2011, however, increasing PR outbreaks caused by an emerging PRV variant have been reported in Bartha-K61-vaccinated swine population on many farms in China. Previously, we showed that the PRV variant TJ strain exhibited enhanced pathogenicity in pigs inoculated via intramuscular route. To develop an animal infection model for accurate evaluation of novel vaccines against the emergent PRV variant, we evaluated the pathogenicity of the PRV TJ strain of different doses in pigs infected via intranasal route. Groups ($n = 5$) of 7-week-old healthy pigs were inoculated intranasally with 10^3, 10^4, 10^5, or 10^6 $TCID_{50}$ (median tissue culture infective dose) PRV TJ strain. Clinical signs, rectal temperature, virus shedding, pathological changes, and seroconversion were monitored. The results showed that the PRV TJ strain induced varied morbidity and mortality (0/5 to 5/5), clinical signs, and tissue lesions, increasingly correlated with the infection doses, and the median lethal dose (LD_{50}) of the virus was determined to be $10^{4.5}$ $TCID_{50}$. Together, this study demonstrates the dose-dependent pathogenicity of the PRV variant via the intranasal route of infection, which provides an ideal animal infection model for evaluation of novel vaccines against the emerging PRV variant.

1. Introduction

Pseudorabies (PR) or Aujeszky's disease (AD), caused by pseudorabies virus (PRV) also known as suid herpesvirus 1 (SuHV-1), is a serious disease of pigs and other animals, leading to significant economic losses to the pig industry in many countries. The disease is characterized by neurological signs, severe respiratory illness, abortions, reduced litter size, and decreased growth rates of survivors, and high morbidity and mortality in newborn piglets (Mettenleiter, 1996; Pomeranz et al., 2005).

PRV belongs to the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family. The viral genome is a linear double-stranded DNA molecule of approximately 145 kb (Szpara et al., 2011). The natural reservoir of PRV is the pig (Keros et al., 2014), although PRV has a broad host range, including most domestic animals such as cattle, sheep, dogs, cats, and goats (Klupp et al., 2004).

By implementing vaccination campaigns with gE-deleted vaccines (such as Bartha-K61) and other measures, PR has been successfully eradicated in many countries and regions (Nauwynck et al., 2007; Müller et al., 2011). Since mid-1980s, Bartha-K61-based vaccines have been widely applied in China, resulting in relatively favorable control of PR (Tong and Chen, 1999). Since 2011, however, increasing PR outbreaks have been reported in Bartha-K61-vaccinated swine population in China, which were shown to be caused by a PRV variant (Luo et al., 2014; Wang et al., 2014; Hu et al., 2015).

The pathogenicity of PRV is variable, depending on the virulence of the virus strain, the age of the pig, and the infection route. Experimentally, the pigs can be infected via intramuscular (i.m.) or/and intranasal (i.n.) route (Maresch et al., 2012; Luo et al., 2014), whereas under natural conditions the oronasal route infection is most common. Previously, we demonstrated that the PRV variant TJ strain of higher dose (10^6 $TCID_{50}$) caused sudden deaths in 7-week-old pigs infected i.m. within 6 days post-infection (dpi), with

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a mortality of 66.7%, while pigs infected with lower dose of the PRV variant were recovered without obvious clinical signs (Wang et al., 2014; Luo et al., 2014). Another study showed that pigs older than 7 weeks infected via i.m. route survived from infection (unpublished data). On the other hand, the PRV TJ strain of 10^6 $TCID_{50}$ caused 100% mortality within 6 dpi in 7-week-old pigs infected via i.n. route. Therefore, the pathogenicity of the PRV variant needs to be further clarified.

The present study was designed to pinpoint the pathogenicity of the PRV TJ variant in pigs in order to develop an animal infection model for accurate evaluation of novel vaccines against the PRV variant.

2. Materials and methods

2.1. Virus strain and cell lines

The PRV TJ strain was isolated from a Bartha-K61-vaccinated pig farm in Tianjin, China (Luo et al., 2014). The virus was propagated in Vero cells and titrated in PK-15 cells, which were grown in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, USA) supplemented with 10% heat-inactivated fetal bovine sera (FBS) (Gibco, USA), 100 μg/ml streptomycin, and 100 IU/ml penicillin at 37 °C and 5% CO_2.

2.2. Experimental animals

Twenty-three 7-week-old healthy pigs (body weight of 7–10 kg) were used in this study. All pigs were tested negative for PRV and anti-PRV antibodies using a PCR method (Luo et al., 2014) and PRV anti-gE or gB antibody detection kits (IDEXX, Westbrook, USA) The pigs were maintained in the animal facility at Harbin Veterinary Research Institute under standard conditions prescribed by the Institutional Guidelines. The study protocol was approved by the Institutional Animal Care and Use Committee. Each group was placed in an individual room.

2.3. Intranasal inoculation of pigs with the PRV TJ strain

The pigs were randomly allocated into five groups. Four groups ($n = 5$) were inoculated via i.n. route with 10^3, 10^4, 10^5, or 10^6 $TCID_{50}$ PRV TJ strain, respectively. The fifth group ($n = 3$) were inoculated with DMEM serving as a negative control. After inoculation, clinical signs and rectal temperatures were recorded daily throughout the experiment. The clinical signs of fever, anorexia, cough, tremor, pruritus, and diarrhea was recorded and scored by a veterinarian based on a standard scoring system. Each parameter was scored from 0 (no signs) to 3 (severe signs).

2.4. Virus isolation

Nasal and rectal swabs were collected daily post-inoculation and subjected to virus isolation as described previously (Wang et al., 2014). Briefly, the samples were immersed in PBS and centrifuged at 2500 × *g* for 10 min. The supernatant was passed through a 0.45-μm filter and inoculated into PK-15 cell monolayers. After a 48-h incubation, the development of cytopathic effects (CPEs) was observed and recorded.

2.5. Pathological examination

At 15 dpi, all pigs were euthanized and subjected to macroscopical and microscopical examinations as described previously (Wang et al., 2014). Briefly, brain, lymph nodes, stomach, heart, tonsils, lungs, and bladder were collected, fixed with buffered 4% formalin and subsequently embedded in paraffin wax. Tissue sections (around 4-μm thick) were prepared and stained with hematoxylin and eosin (H–E) for histopathological examinations. The examinations were evaluated by a board certified veterinary pathologist based on a standard scoring system. Each parameter was scored from 0 (no signs) to 3 (severe signs).

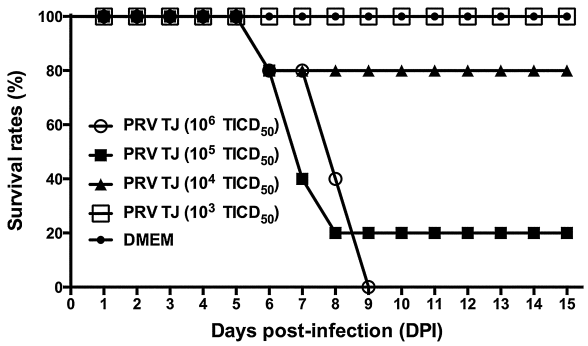

Fig. 1. Survival rates of pigs inoculated with different doses of the PRV TJ strain. Four groups were inoculated i.n. with 10^6, 10^5, 10^4, and 10^3 $TCID_{50}$ PRV TJ strain, respectively. Three non-inoculated pigs served as a negative control.

2.6. Blocking ELISA

Serum samples were collected at 0, 3, 6, 9, 12, and 15 dpi and tested for PRV gE- and gB-specific antibodies by using the IDEXX PRV antibody detection kits according to the manufacturer's instructions.

2.7. Statistical analysis

Data were analyzed using the SPSS 14.0 software. One-way ANOVA followed by Duncan's multiple range tests was used to compare the parameters among the different groups.

3. Results

3.1. Clinical features of infected pigs

The pigs inoculated i.n. with the PRV TJ strain developed varied clinical signs, such as high fever, anorexia, cough, tremor, pruritus, and diarrhea, with the severity correlated with the administered doses (10^3, 10^4, 10^5, or 10^6 $TCID_{50}$). Moreover, the onset of fever varied from 2 dpi (the 10^6 $TCID_{50}$ group) to 4 dpi (the 10^3 $TCID_{50}$ group). Different doses of the virus caused quite different fever incidence, ranging from 26/37 (10^6 $TCID_{50}$), 17/37 (10^5 $TCID_{50}$), 22/57 (10^4 $TCID_{50}$), to 22/75 (10^3 $TCID_{50}$). All the pigs inoculated with the PRV TJ strain showed virus shedding at 2–15 dpi (Table 1). The pigs received 10^3 $TCID_{50}$ did not show obvious clinical signs except transient fever, and all survived from infection. The clinical sign scores were correlated with the inoculation doses of the virus, varying from 76.0 (10^6 $TCID_{50}$) to 1.4 (10^3 $TCID_{50}$) (Table 2). Deaths started at 6 dpi, with survival rates of 0/5, 1/5, 4/5, 5/5, and 5/5 for 10^6, 10^5, 10^4, and 10^3 $TCID_{50}$ groups, respectively (Fig. 1). The median lethal dose (LD_{50}) of the PRV TJ strain was determined to be $10^{4.5}$ $TCID_{50}$.

3.2. Pathological findings in the infected pigs

Pigs died of infection were subjected to macroscopical and microscopical examinations following standard operational procedures, and any observed changes were recorded.

At the pathological examination, various degrees of macroscopical lesions were found in the respiratory system of the PRV-inoculated pigs. In the nasal cavity, larynx and trachea, grade 3, multifocal to coalescing necrotizing epithelial lesions were