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Persistence of porcine rubulavirus in experimentally infected boars

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ABSTRACT

Porcine rubulavirus is the etiological agent of blue eye disease in pigs. In boars, this virus causes orchitis and epididymitis and reduces seminal quality. The objective of this study was to determine the persistence of porcine rubulavirus in experimentally infected boars. Nine 12-month-old boars were infected with 5 ml of the PAC-3 strain of porcine rubulavirus at 1×10^5 $TCID_{50}$/ml and held for 142 days post infection (DPI) to evaluate humoral immune response. The virus was isolated in cell cultures and detected by RT-PCR. Infection with porcine rubulavirus produced clinical signs beginning at 5 DPI. Necropsy results showed that 3 boars had lesions in the testicles and epididymes. Histological analysis showed the characteristic lesions in all infected boars. Porcine rubulavirus antibodies were detected in the second week post infection and increased significantly ($P < 0.05$) over time. Isolation of the virus from semen was achieved between 5 DPI and 48 DPI and from the testicles and epididymes between 64 DPI and 142 DPI. Viral RNA was detected in the serum between 2 DPI and 64 DPI and in the semen until 142 DPI. These results confirm that the RNA of the porcine rubulavirus persists in the semen and that this virus remains in the reproductive tract for prolonged periods of infection. Semen of persistently infected boars, therefore, represents an important source of the virus and a risk factor for the spread of blue eye disease in swine populations.

1. Introduction

Porcine rubulavirus (PoRV) is the etiological agent of blue eye disease (BED) in pigs (Stephano et al., 1988). The virus is an enveloped, single-stranded, negative-sense RNA virus that belongs to the *Paramyxoviridae* family of the genus *Rubulavirus* (Lamb et al., 2005; Moreno-Lopez et al., 1986; Wang et al., 2007). BED has been diagnosed exclusively in Mexico, where it persists as an endemic disease in the major swine-producing zones, especially in the central and west-central regions of the country (Escobar-Lopez et al., 2012; Kirkland and Stephano, 2006; Morilla et al., 2002). The clinical signs associated with BED depend on the age of the pigs. In piglets, the infection has been associated with neurological disorders and corneal opacity (Moreno-Lopez et al., 1986; Stephano et al., 1988), while in adults, it has been associated with reproductive failure. Increased numbers of sows returning to oestrus and an increased incidence of stillbirths and mummified foetuses have been observed (Hernandez-Jauregui et al., 2004; Kirkland and Stephano,

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2006). In boars, the documented signs of infection include orchitis, epididymitis and reduced seminal quality that can lead to permanent infertility. In experimentally infected adult animals, the reproductive tract organs were found to be sites of porcine rubulavirus replication (Ramirez-Mendoza et al., 1997). Studies have also suggested that porcine rubulavirus may be excreted in the semen during the clinical phase of the disease (Solis et al., 2007). Although the persistence of porcine rubulavirus has been proven by the detection of viral RNA in the central nervous system, lymph nodes and reproductive tract of boars (Cuevas et al., 2009; Wiman et al., 1998), viral RNA has not been detected in semen, and the virus has not been isolated during the phase of persistent infection. Hence, the objective of this study was to determine whether the porcine rubulavirus or its viral RNA persists in experimentally infected boars during prolonged periods of infection.

2. Materials and methods

2.1. Cells and virus

The PAC-3 strain of porcine rubulavirus (Jalisco/1992; GenBank access number: EF413173) was replicated in baby hamster kidney cell lines (BHK-21) using Dulbecco's Modified Eagle's Medium/Nutrient mixture F12 Ham (DMEM; Sigma–Aldrich, St. Louis, MO) supplemented with 5% foetal bovine serum (FBS) and 500 mg ml^{-1} of gentamicin. The virus stock was titrated by 50% tissue culture infectious dose per millilitre ($TCID_{50}$) using the Reed and Muench method. The PAC-3 strain has been shown to cause reproductive failure in sows and testicular and epididymal damage in boars (Ramirez-Mendoza et al., 1997).

2.2. Animals and experimental design

Nine 12-month-old York × Landrace boars were obtained from a porcine rubulavirus-free farm. When the boars were selected, they were subjected to serological examinations (haemagglutination inhibition and virus neutralisation) to confirm their seronegativity; likewise, RT-PCR of peripheral blood mononuclear cells and of semen was performed during the four adaptation weeks before infection. The boars were held in experimental isolation units at the *Departamento de Medicina y Zootecnia de Cerdos* of the *Facultad de Medicina Veterinaria y Zootecnia* at *Universidad Nacional Autónoma de México*. All boars were fed a commercial diet and received water *ad libitum*. Inoculation was performed by intranasal instillation with 2.5 ml 1×10^5 $TCID_{50}$/ml porcine rubulavirus/PAC-3 deposited into each nostril on day zero. All procedures and the experimental protocol were approved by the Institutional Experimental Animal Care Sub-committee of the *Universidad Nacional Autónoma de México*.

2.3. Clinical observation

During the adaptation period and after experimental infection, the boars were evaluated daily for clinical signs and behavioural changes. The physical exam included rectal temperature measurement, morphometric testicular measurement with a vernier caliper, and direct palpation of the epididymis. All observations were recorded for a period of 142 days post infection (DPI).

2.4. Necropsy and sample collection

Whole ejaculates were collected once a week for 2 weeks before infection and until the end of the experiment (at 20 weeks). Semen collection was performed in a special pen using a dummy; the semen was obtained using the gloved hand technique, and ejaculates were collected in sterile recipients. Blood samples were collected from the jugular vein in Vacutainer® tubes concurrently with the ejaculate collections; serum was obtained by centrifugation. Boars were euthanised at 3 different points during the experiment: one at 64 DPI (10 weeks), 4 at 110 DPI (16 weeks) and the remaining 4 boars at 142 DPI (20 weeks). A macroscopic examination of the reproductive tract was performed at necropsy, and testicle and epididymis (head and tail) samples were collected. Sections of tissues were fixed in 10% neutral buffered formalin for histopathological examination. All fresh samples were stored in liquid nitrogen until use.

2.5. Histopathological evaluation

The formalin-fixed tissue samples were embedded in paraffin wax, sectioned and stained with haematoxylin–eosin. These sections were evaluated by light microscopy for histopathological changes.

2.6. Serology

To detect an antibody response, a haemagglutination inhibition (HI) assay was performed as previously described, using eight haemagglutination units of PAC-3 and 0.5% bovine red blood cells (Ramirez et al., 1996). A virus neutralisation (VN) assay was performed with 300 $TCID_{50}$ PAC-3 on Madin-Darby canine kidney cell lines (MDCK). Double serial dilutions of the sera were performed for both tests. The titre of the haemagglutination inhibition antibody was expressed as the maximum dilution at which the serum was able to inhibit the haemagglutination activity of the porcine rubulavirus. The neutralising antibody titration was expressed as the maximum dilution at which the serum was able to neutralise the replication of the porcine rubulavirus in cell cultures, as reflected by the haemagglutination action (Hernandez et al., 1998). For statistical purposes, the titres were transformed into $\log_2$ values.

2.7. Virus isolation

Viral isolation was performed using cell cultures. The semen samples were diluted 1:5 in DMEM without supplements and then centrifuged at 3000 × *g* for 10 m. The supernatants were collected and filtered through 0.22 μm sterile nitrocellulose membranes. MDCK cells were cultured in 96-well plates, inoculated at 80% cell confluence and incubated for 60 min at 37 °C. The