CLINICAL AND VACCINE IMMUNOLOGY, July 2011, p. 1091–1096 Vol. 18, No. 7
1556-6811/11/$12.00 doi:10.1128/CVI.05027-11

Effects of an Inactivated Porcine Circovirus Type 2 (PCV2) Vaccine on PCV2 Virus Shedding in Semen from Experimentally Infected Boars▽

Hwi Won Seo,[1]† Kiwon Han,[1]† Duyeol Kim,[1]† Yeonsu Oh,[1] Ikjae Kang,[1] Changhoon Park,[1] Hyun Jang,[2] and Chanhee Chae[1]*

Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea,[1] and Komipharm International Company Ltd., 1 Ra-107 Shiwha Industrial Complex, Shiheung-shi, Kyongki-do, Republic of Korea[2]

Received 14 March 2011/Returned for modification 4 April 2011/Accepted 6 May 2011

The objective of the present study was to determine the effect of an inactivated porcine circovirus type 2 (PCV2) vaccine on PCV2b virus shedding in the semen of experimentally infected boars by measuring the immunological response and the PCV2b DNA load in blood and semen. Twelve boars were randomly divided into three groups. The boars in group 1 ($n = 4$) were immunized with an inactivated PCV2 vaccine and were challenged with PCV2b. The boars in group 2 ($n = 4$) were only challenged with PCV2b. The boars in group 3 ($n = 4$) served as negative controls. The number of PCV2 genome copies of PCV2 in the serum and semen were significantly lower in vaccinated challenged boars than in nonvaccinated challenged boars at 7, 10, 14, 21, 32, 35, 42, 49, and 60 days postinoculation. The number of PCV2b genomes in the semen correlated with the number of PCV2b genomes in the blood in both vaccinated challenged ($R = 0.714$) and nonvaccinated challenged ($R = 0.861$) boars. The results of the present study demonstrate that the inactivated PCV2 vaccine significantly decreases the amount of PCV2b DNA shedding in semen from vaccinated boars after experimental infection with PCV2b.

Porcine circovirus type 2 (PCV2) is associated with a number of diseases and syndromes collectively referred to as porcine circovirus-associated disease (PCVAD), which includes postweaning multisystemic wasting disease (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), reproductive failure, porcine respiratory disease complex (PRDC), and exudative epidermitis (3).

Several commercial PCV2 vaccines are currently available in the global market. All PCV2 vaccines have been administered to either sows or piglets (2, 5, 16, 18). Commercial PCV2 vaccines are able to reduce the PCV2 load in the serum and reduce PCV2 shedding in nasal and fecal samples in conventional pigs (5, 15, 16, 18). Recently, it has also been reported that vaccination against PCV2 in naturally infected boars can decrease the duration of viral shedding in semen (1). These data strongly imply that vaccination against PCV2 may reduce the subsequent shedding of PCV2 in the semen of boars. Therefore, the objective of the present study was to determine the effect of PCV2 vaccination on the shedding of the PCV2 virus in the semen of experimentally infected boars.

MATERIALS AND METHODS

Commercial vaccine and PCV2 inoculum. The commercial inactivated tissue homogenate PCV2 vaccine based on PCV2 genotype 2b, CircoPrime (Komipharm International Company Ltd., Shiheung-shi, Kyongki-do, Republic of Korea), was used in this study. It was administered intramuscularly in two 2.0-ml doses separated by 3 weeks.

The PCV2 strain (PCV2 genotype b) isolated from the superficial inguinal lymph node of a field case of PMWS was used as the inoculum. The virus was propagated in PCV-free PK15 cells to a titer of 10^5 50% tissue culture infective doses ($TCID_{50}$s)/ml.

Experimental design. At 8 months of age, 12 purebred, male, Landrace pigs were purchased from a commercial farm. All boars were negative for PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV), according to routine serological testing performed prior to delivery and again on arrival.

All boars were housed individually in an environmentally controlled building with pens over completely slatted floors throughout the experiment. To avoid environmental contamination, the building was completely emptied, cleaned three times with hot (>95°C) water, and disinfected with a 2% potassium peroxymonosulfate and sodium chloride-based product (Virkon S; Antec International, Sudbury, Suffolk, United Kingdom) for 3 days. The building was empty for an additional 21 days before the boars were introduced, and each boar was housed separately within the facility.

The boars were randomly divided into three groups. The boars in group 1 (T01; $n = 4$) were immunized with two 2.0-ml doses of the inactivated PCV2 vaccine at a 3-week interval, and then, at 3 weeks after the second vaccination, the boars were intranasally inoculated with PCV2b (3 ml) with an infectious titer of $10^{4.5}$ $TCID_{50}$s per ml. The boars in group 2 (T02; $n = 4$) were intranasally inoculated with PCV2b (3 ml) with an infectious titer of $10^{4.5}$ $TCID_{50}$s per ml. The concentration of PCV2b ($10^{4.5}$ $TCID_{50}$s per ml) for inoculation was similar to that used in a previous study to allow comparison (13). The boars in group 3 (T03; $n = 4$) served as negative controls.

Serology. Blood samples from each pig were collected by jugular venipuncture at −42, −21, 0, 14, 21, 28, 35, 42, 49, and 60 days postinoculation (dpi), and the serum samples were tested using a commercial PCV2 enzyme-linked immunosorbent assay IgG kit (Ingezim Circovirus IgG; Ingenasa, Madrid, Spain).

Quantification of PCV2 DNA. DNA was extracted from semen (raw) and serum samples collected at −42, −21, −7, 0, 4, 7, 10, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, 56, and 60 dpi using a QIAamp DNA minikit (Qiagen, Valencia, CA) as described previously (19). DNA extracts were used to quantify the PCV2 genome DNA copy numbers by real-time PCR as described previously (6).

Standard curve. To construct a standard curve, real-time PCRs were performed in quadruplicate in two different assays: (i) 10-fold serial dilutions of the PCV2 plasmid were used as the standard, with concentrations ranging from 10^{10} to 10^2 copies/ml, and (ii) 10-fold serial dilutions of PCV2b cultured in PK-15 cells free of PCV1 were used at concentrations ranging from $10^{4.5}$ $TCID_{50}$s/ml to $10^{-3.5}$ $TCID_{50}$s/ml. The PCV2 plasmid was prepared as described previously (6).

* Corresponding author. Mailing address: Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea. Phone and fax: 82-2-880-1277. E-mail: swine@snu.ac.kr.
† These authors contributed equally to this work.
▽ Published ahead of print on 25 May 2011.