Vaccine 32 (2014) 4342–4348

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Commercial PCV2a-based vaccines are effective in protecting naturally PCV2b-infected finisher pigs against experimental challenge with a 2012 mutant PCV2

Tanja Opriessnig[a,b,*], Priscilla F. Gerber[b], Chao-Ting Xiao[b], Patrick G. Halbur[b], Shannon R. Matzinger[c], Xiang-Jin Meng[c]

[a] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[c] Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 13 May 2014
Accepted 2 June 2014
Available online 11 June 2014

Keywords:
Genotypes
Porcine circovirus
PCV2
Mutant PCV2
Vaccine efficacy
Vaccination

ABSTRACT

Current commercial PCV2 vaccines are all based on PCV2a and have been shown to be effective in reducing PCV2a and PCV2b viremia and PCV2-associated lesions and disease. The recent emergence of novel mutant PCV2 (mPCV2) strains and linkage of mPCV2 with cases of porcine circovirus associated disease (PCVAD) in vaccinated herds have raised concerns over emergence of vaccine-escape mutants and reduced efficacy of PCV2a-based vaccines. The aim of this study was to determine the ability of three commercial PCV2a-based vaccines administered in the presence of an ongoing PCV2b infection and passively-acquired anti-PCV2 antibodies to protect conventional pigs against experimental challenge with mPCV2 at 11 weeks of age. Fifty naturally PCV2b-infected 2-week-old pigs were divided into five treatment groups with 10 pigs each. Pigs were unvaccinated (positive and negative controls) or vaccinated at 3 (VAC-A, VAC-B, VAC-C) and at 5 weeks of age (VAC-C). At 11 weeks of age, all pigs except the negative controls were challenged with a 2012 U.S. strain of mPCV2. The experiment was terminated 21 days after challenge. Under the conditions of this study, vaccinated pigs were protected against PCV2 viremia and lesions whereas non-vaccinated pigs were not. Moreover, concurrent PCV2b and mPCV2 infection was demonstrated in all positive controls and 3/10 had microscopic lesions consistent with PCVAD while negative controls infected with PCV2b alone did not develop PCVAD. The results indicate that concurrent PCV2b/mPCV2 infection can trigger PCVAD development and that commercial vaccines are effective in protecting conventional pigs against emerging mPCV2 strains.

1. Introduction

In response to the severe porcine circovirus associated disease (PCVAD) outbreaks in North America during 2005–2006, several inactivated or subunit PCV type 2 (PCV2) vaccines became commercially available [1]. These vaccines are all based on the PCV2a genotype and in general continue to be effective against both PCV2a and PCV2b based on experimental inoculations [2,3] and under field conditions [4–7]. Because of their efficacy, PCV2 vaccines have become widely used worldwide with an estimated 99% of all growing U.S. pigs being vaccinated.

In 2012, a novel mutant strain of PCV2 (mPCV2) was discovered in pigs in the United States [8] and often was associated with PCVAD-induced increased mortality in late finisher pigs [9]. This particular mPCV2 strain is characterized by several unique amino acid mutations throughout the capsid and by the addition of a lysine residue at the end of ORF2 due to a stop codon mutation [10]. While the mPCV2 is apparently new to the U.S., closely-related virus strains have been identified in China since 2002 [11,12]. Interestingly, in some geographic areas in China, the mPCV2 strains seem to have replaced the traditional PCV2b isolates [13].

It is well recognized that commercial vaccines do not completely eliminate PCV2 viremia [14,15] and that occasionally PCVAD outbreaks in vaccinated herds occur [16]. There are increasing concerns that the currently available commercial vaccines may not

* Corresponding author at: The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, UK.
Tel.: +44 (0)131 651 9177.
E-mail address: Tanja.Opriessnig@roslin.ed.ac.uk (T. Opriessnig).

http://dx.doi.org/10.1016/j.vaccine.2014.06.004
0264-410X/

Table 1
Experimental design.

Group designation	Number of pigs	Vaccination		Challenge 11
		3[a]	5	
Negative controls	10	–	–	–
Positive controls	10	–	–	mPCV2
VAC-A[b]	10	1 ml	–	mPCV2
VAC-B[d]	10	2 ml		mPCV2
VAC-C[c]	10	2 ml	2 ml	mPCV2

[a] Age in weeks.
[b] Ingelvac® CircoFLEX (Boehringer-Ingelheim Vetmedica).
[c] Circumvent® PCV (Merck Inc.).
[d] Fostera™ PCV (Zoetis Inc.).

be fully capable of protecting against emerging mPCV2s and that the large-scale massive PCV2 vaccination program may perhaps promote the development of vaccine-escape variants. In a recent pilot study using PCV2 naïve pigs, it was demonstrated that two-dose vaccination using a commercial PCV2a-based product or an experimental mPCV2-based vaccine protected pigs against challenge with mPCV2 [17]. However, there is continuing concern about active PCV2 infections and varying levels of passively-acquired antibodies at the time of PCV2 vaccination which both may interfere with vaccine efficacy and duration of immunity contributing to the occurrence of PCVAD in the later finisher period under field conditions. The objective of this study was to compare the ability of commercial PCV2a-based vaccines to protect naturally PCV2b-infected conventional pigs against mPCV2 challenge at 11 weeks of age.

2. Materials and methods

2.1. Animals, housing, and experimental design

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. Fifty, 2-week-old, colostrum-fed, arbitrarily selected crossbred pigs from a source herd negative for porcine reproductive and respiratory syndrome virus and influenza A virus were blocked by anti-PCV2 IgG ELISA sample-to-positive (*S*/*P*) ratio and randomly assigned to one of five groups with 10 pigs in each group (Table 1). The pigs were moved into a separate room on the farm of origin and were commingled regardless of vaccination status. At 3 weeks of age, VAC-A, VAC-B, and VAC-C pigs were vaccinated according to Table 1. At 5 weeks of age, the VAC-C pigs were revaccinated as recommended by the vaccine manufacturer. Negative and positive control pigs were not vaccinated. At 10 weeks of age, all pigs were transferred to the Iowa State University research facility. At arrival, negative controls were placed in a separate room and the remaining pigs were arbitrarily assigned to one of two rooms with four pens in each room. Each pen was equipped with one nipple drinker and one self-feeder. All groups were fed *ad libitum* with a balanced, age-appropriate, pelleted feed ration (Nature's Made, Heartland Co-op, IA, USA). Challenge with mPCV2 was done at 11 weeks of age and all pigs were humanely euthanized and necropsied at 14 weeks of age.

2.2. Vaccination

The pigs were vaccinated intramuscularly in the right neck at 3 weeks of age with 1 ml of Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica; Lot No. 309–698; VAC-A), 2 ml of Fostera™ PCV (Zoetis Inc.; Lot No. 1201402A; VAC-B), or 2 ml of Circumvent® PCV (Merck Inc.; Lot No. 02132947; VAC-C). The VAC-C group was revaccinated at 5 weeks of age with 2 ml of the vaccine.

2.3. Challenge

The full-length genome of the mutant PCV2b isolate JX535296 was amplified by PCR using previously described primers from DNA extracted from a 2012 lung homogenate of an Iowa pig with severe PCVAD [8,18]. The PCR product was blunt ligated to the pCR® Blunt II-TOPO® vector using the Zero Blunt® TOPO® PCR Cloning Kit (Life Technologies, Carlsbad, CA, USA) per the manufacturer's instructions. After isolation of the full-length virus genome from the mPCV2 clone, the full-length mPCV2 genome was concatemerized and subsequently transfected into PK-15 cells to generate an infectious mPCV2 stock [19]. The virus stock was grown to a final titer of $10^{3.66}$ 50% tissue culture infectious dose ($TCID_{50}$) per ml.

To further confirm the infectivity of the mPCV2 challenge virus stock, it was used to inoculate three separate PCV2 naïve 3-week-old pigs. All three pigs were viremic at 7 days after inoculation and seroconverted by dpc 21 (data not shown). The presence of authentic mPCV2 in these pigs was confirmed by further sequencing the virus recovered from infected pigs, thus confirming the infectivity of the mPCV2 virus stock. At 11 weeks of age, positive control, VAC-A, VAC-B, and VAC-C pigs (Table 1) received 5 ml of the mPCV2 challenge virus stock intranasally by slowly dripping 2.5 ml in each nostril.

2.4. Average daily weight gain, clinical observations, and serum collections

All pigs were weighed at −63 days post challenge or dpc (before vaccination, 2 weeks of age), at 0 dpc (challenge, 11 weeks of age) and at 21 dpc (necropsy, 14 weeks of age). The average daily weight gain was calculated before (−63 to 0 dpc) and after mPCV2 challenge (0–21 dpc). After mPCV2 challenge, all animals were examined daily for signs of illness such as lethargy, respiratory disease, inappetance, and lameness. At −63 dpc and again at 0, 7, 14 and 21 dpc, blood was collected from all pigs and serum was stored at −80 °C until testing.

2.5. Laboratory testing

2.5.1. Serology

Serum samples collected at −63, 0, 7, 14, and 21 dpc were tested for the presence of anti-PCV2 IgG antibodies by an in house ORF2-based ELISA [20].

2.5.2. DNA extraction

Total nucleic acids were extracted from serum samples and tissue homogenates using the MagMax™ Pathogen RNA/DNA Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and an automated DNA/RNA extraction system (Thermo Scientific Kingfisher® Flex, Thermo Fisher Scientific, Pittsburgh, PA, USA) according to the instructions of the manufacturer.

2.5.3. Detection and quantification of PCV2 DNA

All DNA extracts were tested for the presence of PCV2 DNA by a quantitative real-time PCR assay targeting a conserved region in ORF1 as described previously [21,22] with the following modifications: a commercially available master mix (TaqMan® Universal PCR Master Mix, Applied Biosystems) was used, the reaction volume was 25 μl and the thermal cycler conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. Samples were considered negative when no signal was observed within the 40 amplification cycles. Five serial dilutions of a PCV2 genomic DNA clone (10^5–10^9 copies/ml) were used to generate a standard curve with a correlation coefficient of more than 0.99 [21].