Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

A commercial porcine circovirus (PCV) type 2a-based vaccine reduces PCV2d viremia and shedding and prevents PCV2d transmission to naïve pigs under experimental conditions

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

[a] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, Scotland, UK
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA
[c] College of Biology, Hunan University, Changsha, China
[d] 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 23 August 2016
Received in revised form 17 November 2016
Accepted 24 November 2016
Available online xxxx

Keywords:
Porcine circovirus
PCV2
PCV2d
Vaccination
Vaccine efficacy
Transmission

ABSTRACT

Porcine circovirus type 2 (PCV2) vaccination has been effective in protecting pigs from clinical disease and today is used extensively. Recent studies in vaccinated populations indicate a major PCV2 genotype shift from the predominant PCV2 genotype 2b towards 2d. The aims of this study were to determine the ability of the commercial inactivated PCV2a vaccine Circovac® to protect pigs against experimental challenge with a 2013 PCV2d strain and prevent transmission. Thirty-eight pigs were randomly divided into four groups with 9–10 pigs per group: NEG (sham-vaccinated, sham-challenged), VAC (PCV2a-vaccinated, sham-challenged), VAC + CHAL (PCV2a-vaccinated and PCV2d-challenged), and CHAL (sham-vaccinated, PCV2d-challenged). Vaccination was done at 3 weeks of age using Circovac® according to label instructions. The CHAL and VAC + CHAL groups were challenged with PCV2d at 7 weeks of age and all pigs were necropsied 21 days post-challenge (dpc). The VAC-CHAL pigs seroconverted to PCV2 by 21 days post vaccination (dpv). At PCV2d challenge on 28 dpv, 3/9 VAC and 1/9 VAC + CHAL pigs were seropositive. NEG pigs remained seronegative for the duration of the study. Vaccination significantly reduced PCV2d viremia (VAC + CHAL) at dpc 14 and 21, PCV2d fecal shedding at dpc 14 and 21 and PCV2d nasal shedding at dpc 7, 14 and 21 compared to CHAL pigs. Vaccination significantly reduced mean PCV2 antigen load in lymph nodes in VAC + CHAL pigs compared to CHAL pigs. When pooled serum or feces collected from VAC + CHAL and CHAL pigs at dpc 21 were used to expose single-housed PCV2 naïve pigs, a pooled fecal sample from CHAL pigs contained infectious PCV2 whereas this was not the case for VAC + CHAL pigs suggesting reduction of PCV2d transmission by vaccination. Under the study conditions, the PCV2a-based vaccine was effective in reducing PCV2d viremia, tissue loads, shedding and transmission indicating that PCV2a vaccination should be effective in PCV2d-infected herds.

1. Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped, circular-arranged, single-stranded DNA virus that belongs to the *Circoviridae* family [1]. PCV2 is ubiquitous and very resistant to disinfection [2] and most pigs get exposed to PCV2 during their life. In growing pigs, PCV2-infection can be associated with a variety of clinical manifestations commonly summarized as PCV2 associated disease (PCVAD) including systemic illness, enteritis and pneumonia [3]. Porcine dermatitis and nephropathy syndrome (PDNS) has also been linked to PCVAD [4,5], although definitive experimental proof is still lacking. In addition to PCVAD, PCV2 infection can result in subclinical disease for extended periods of time, which can have a varying impact on pork production [6,7]. Non-specific clinical signs including reduced weight gain associated with subclinical PCV2 infection are thought to occur due to the effect of PCV2 on the immune system [8].

PCV2 can be classified in five different genotypes including PCV2a, PCV2b, PCV2c, PCV2d and PCV2e of which PCV2a is the oldest [9,10]. PCV2c has only been identified in archived pig tissues

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

http://dx.doi.org/10.1016/j.vaccine.2016.11.085
0264-410X/

from Denmark [11] and a recent feral pig sample from Brazil [12] and is considered of minor importance. Around 2003 a major genotype shift occurred from PCV2a to PCV2b [11]. Severe PCV2 epidemics linked to PCV2b introduction occurred in North America during 2005/2006 [13] and subsequently led to introduction and large scale usage of PCV2 vaccines in pigs. Today PCV2 vaccination has become a standard management tool in most pig producing areas [14]. Supported by numerous field and experimental trials, PCV2 vaccination has been proven to reduce PCV2 infection, viremia and lesions and increases average daily weight gain (ADG) compared to non-vaccinated pigs [14]. Development of most commercial PCV2 vaccines occurred between 1999 and 2005 when little information on PCV2 genotypes was available and PCV2a was the predominant PCV2 strain at the time. Therefore all major PCV2 vaccines available to date are based on PCV2a [3]. Nevertheless, PCV2a vaccines have been shown to protect pigs against PCV2b challenge in several independent studies [15,16].

Previously it has been determined that PCV2 has a high mutation rate similar to RNA viruses [17] which may further facilitate rapid emergence and transmission of unique PCV2 genotypes. Furthermore, pigs are often co-infected with multiple PCV2 strains [18,19]. Since the beginning of this decade a newly recognized genotype, PCV2d, emerged in essentially all large pig populations in North America, South America, Europe and Asia [9,20]. Moreover, several studies indicate that PCV2d is becoming the predominant strain in the global pig population replacing PCV2a and PCV2b [9,21]. Frequently, the presence of PCV2d has been linked to PCVAD outbreaks in PCV2-vaccinated herds [22–24] raising concerns that PCV2 vaccines based on PCV2a strains may not provide sufficient protection against PCV2d strains. The objectives of this study were to determine the ability of a commercial inactivated PCV2a vaccine to protect conventional pigs against experimental challenge and to prevent transmission of a 2013 PCV2d to naïve contact pigs.

2. Materials and methods

2.1. Ethical statement

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (Approval number: 11-14-7900-S).

2.2. Animals, housing, and experimental design

Two-week-old, colostrum-fed, crossbred pigs, from a high health commercial breeding herd free of *Mycoplasma hyopneumoniae*, influenza A virus and porcine reproductive and respiratory syndrome virus (PRRSV) and with low PCV2 antibody titers in a portion of the dams and without active PCV2 circulation as evidenced by regular PCV2 PCR testing on pooled serum samples, were purchased for this study. For the main study 38 pigs were randomly assigned to one of four rooms and groups with 9–10 pigs in each group (Table 1). For the contact exposure part of the study, 14 age-matched contact pigs were group-housed in a different room until 7 weeks of age. At that point the contact pigs were moved to individual rooms and were single housed (Fig. 1). Each room contained one pen with one nipple drinker and one self-feeder. All groups were fed *ad libitum* with a balanced, age-appropriate, pelleted feed ration. The experimental design and sample collections are summarized in Fig. 1. Blood was collected in serum separator tubes (BD Vacutainer SST, REF 367088; Fisher Scientific, Pittsburgh, PA, USA), centrifuged at 3000*g* for 10 min at 4 °C, and the serum was stored at −80 °C until testing. Nasal and rectal swabs were collected using polyester swabs and were stored in 5 ml plastic tubes containing 1 ml of sterile saline solution at −80 °C until testing.

2.3. Vaccination

At 3 weeks of age (dpv 0 or dpc −28), the VAC and VAC + CHAL pigs were vaccinated intramuscularly in the left neck with 0.5 ml of Circovac® (Merial; Lot No. L404456) as recommended by the manufacturer (Table 1). Similarly, the CHAL and NEG pigs were sham-vaccinated intramuscularly in the left neck with 0.5 ml saline.

2.4. Challenge

PCV2d isolate JX535296 [22,25] was grown to a final titer of $10^{4.33}$ 50% tissue culture infectious dose ($TCID_{50}$) per ml. At 7 weeks of age (dpv 28 or dpc 0), CHAL and VAC-CHAL pigs (Table 1) received 4.5 ml of the PCV2d challenge virus stock intranasally by slowly dripping 2.25 ml in each nostril. Pigs in the VAC and NEG groups were sham-inoculated with 4.5 ml saline, which was also given intranasally.

2.5. Contact pig exposure

Two serum pools were generated by combining serum samples from all VAC-CHAL or all CHAL pigs collected at day post-challenge (dpc) 21. Once combined, 3 ml of the VAC-CHAL dpc 21 serum pool were administered to contact pigs 10, 11, 12 (Fig. 1) by the intramuscular route at day post-exposure (dpe) 0. Similarly, contact pigs 4, 5 and 6 received 3 ml of the CHAL dpc serum pool by the intramuscular route at dpe 0. Fecal material collected on dpc 21 from VAC-CHAL pigs was diluted in phosphate buffered saline (PBS) and contact pigs 7, 8 and 9 each received 8 ml of fecal suspension by the oral route while 8 ml of fecal suspension collected on dpc 21 from CHAL pigs were administered orally to contact pigs 1, 2 and 3. Contact pigs 13 and 14 served as non-infected negative controls (Fig. 1).

2.6. Average daily weight gain and clinical observations

All pigs in the main study were weighed at 3 weeks of age (dpv 0 or dpc −28), at 7 weeks of age (dpv 28 or dpc 0) and at 10 weeks of age (dpc 21; Fig. 1). The average daily weight gain

Table 1
Experimental groups, treatments at different days post PCV2d challenge (dpc) and average daily weight gain (ADG).

Group designation	Number of pigs	Vaccination	Challenge	ADG[a]	
		dpc −28	*dpc 0*	*Vaccination to challenge*	*Challenge to necropsy*
NEG	10	Saline	Saline	463.3 ± 25.2	795.4 ± 40.4
VAC	9	Circovac®	Saline	351.6 ± 29.0	744.7 ± 46.6
VAC + CHAL	9	Circovac®	PCV2d	426.5 ± 21.6	774.0 ± 46.6
CHAL	10	Saline	PCV2d	412.7 ± 26.0	726.0 ± 49.5

[a] Data presented as group mean ADG in grams ± SEM.

Please cite this article in press as: Opriessnig T et al. A commercial porcine circovirus (PCV) type 2a-based vaccine reduces PCV2d viremia and shedding and prevents PCV2d transmission to naïve pigs under experimental conditions. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.11.085