The Veterinary Journal 200 (2014) 65–70

Contents lists available at ScienceDirect

The Veterinary Journal

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

Clinical, virological, immunological and pathological evaluation of four porcine circovirus type 2 vaccines

Hwi Won Seo, Kiwon Han, Changhoon Park, Chanhee Chae *

Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Republic of Korea

ARTICLE INFO

Article history:
Accepted 7 February 2014

Keywords:
Porcine circovirus (PCV) type 2
PCV-associated disease
Post-weaning multisystemic wasting syndrome
Vaccine

ABSTRACT

The objective of this study was to rigorously compare the efficacy of four porcine circovirus type 2 (PCV2) vaccines of varying antigen type and dose under experimental conditions based on well-defined clinical (average daily weight gain [ADWG]), virological (evidence of viraemia), immunological (presence of PCV2-specific neutralising antibodies [NA], interferon-γ-secreting cells [IFN-γ-SCs], and $CD3^+$ and $CD4^+$ T cell subsets), and pathological (lymphoid lesion and PCV2 antigen score) criteria. A total of 60, 3-week old piglets were assigned to six groups of 10/group and were vaccinated either with 1/4 commercially available one-dose vaccines or were not vaccinated. At 7 weeks of age, vaccinated and control animals were inoculated intranasally with 2 mL of PCV2b. All pigs were euthanased and subjected to post-mortem examination at 25 weeks of age.

From 9 to 16 weeks of age, the ADWG of vaccinated animals was significantly higher than that of non-vaccinates. Significant ($P < 0.05$) differences were observed between vaccinated and positive control groups in the quantity of log-transformed PCV2b DNA in the blood and nasal swabs, log-transformed NA titres, and PCV2-specific IFN-γ-SCs at 0, 7, 14, 21, and 42 days post challenge (dpc). The proportion of $CD4^+$ cells at 7 and 14 dpc was also significantly different between vaccinated and control pigs ($P < 0.05$). The histopathological lesions and PCV2-antigen scores in the lymph nodes were significantly lower ($P < 0.05$) in vaccinated animals. All four vaccines were found to be highly efficacious in controlling experimental PCV2 challenge based on this range of criteria.

© 2014 Elsevier Ltd. All rights reserved.

Introduction

Porcine circovirus type 2 (PCV2) is the main aetiological agent associated with diseases collectively termed porcine circovirus-associated disease (PCVAD) (Chae, 2004, 2005). Of these conditions, post-weaning multisystemic wasting syndrome (PMWS) is considered the most important economically (Chae, 2005). Since PCV2 vaccines were first launched onto the global market in 2006, vaccination has become a major tool for the control of PCVAD. Commercial PCV2 vaccines have been shown to be highly effective in reducing and preventing PCVAD under both experimental and field conditions (Fort et al., 2008, 2009b; Kixmoller et al., 2008; Opriessnig et al., 2009; Segalés et al., 2009; Shen et al., 2010; Martelli et al., 2011; Fraile et al., 2012; Seo et al., 2012).

One of the most important criteria when evaluating the efficacy of such vaccines is the reduction in viraemia, as this plays a central role in the development of PCVAD (Chae, 2012): the level of viraemia is used to categorise PCV2-infected pigs as subclinically infected, and PCVAD-positive, respectively (Liu et al., 2000; Brunborg et al., 2004; Olvera et al., 2004; Segalés et al., 2005). Measurement of viral load can also be used as a parameter to evaluate the efficacy of PCV2 vaccines: reduced viraemia correlates with the presence of PCV2-neutralising antibodies (NA), and interferon-γ-secreting cells (IFN-γ-SCs) (Meerts et al., 2005, 2006; Fort et al., 2007, 2009a). However, only one study has assessed if currently available PCV2 vaccines induce specific IFN-γ-SCs under experimental conditions (Fort et al., 2009b).

Currently, four one-dose PCV2 vaccines are available commercially in Korea. These include two subunit vaccines based on the capsid protein expressed in a baculovirus system, and two inactivated vaccines based on PCV2 or on chimeric PCV1-2. Previous studies have compared virological and pathological outcomes following vaccination with three PCV2 vaccines registered for use in the in USA (Opriessnig et al., 2009; Shen et al., 2010). The objective of the present study was to compare the efficacy of four such PCV2 vaccines based on the evaluation of clinical, virological, immunological, and pathological criteria.

* Corresponding author. Tel.: +82 2 880 1277.
E-mail address: swine@snu.ac.kr (C. Chae).

http://dx.doi.org/10.1016/j.tvjl.2014.02.002
1090-0233/© 2014 Elsevier Ltd. All rights reserved.

Table 1
Efficacy of four commercially-available porcine circovirus type 2 (PCV2) vaccines (Fostera, Circovac, Circoflex, and Porcillis) assessed using the following criteria: average daily weight gain (ADWG), evidence of viraemia and nasal shedding, as well as histopathological lesion, and immunohistochemical viral antigen scores. Dpc, days post challenge.

		Vaccine treatment groups					
		Fostera	Circovac	Circoflex	Porcilis	Positive controls	Negative controls
ADWG (weeks)	3–7	329 ± 25	322 ± 26	324 ± 22	332 ± 28	326 ± 25	331 ± 31
	7–9	706 ± 33	701 ± 41	711 ± 36	701 ± 39	691 ± 35	709 ± 38
	9–16	751 ± 35[a]	756 ± 28[a]	749 ± 33[a]	743 ± 38[a]	706 ± 27[b]	748 ± 34[a]
	16–25	712 ± 41	700 ± 34	704 ± 29	696 ± 38	687 ± 32	706 ± 33
	3–25	654 ± 33	649 ± 37	650 ± 29	645 ± 41	627 ± 35	652 ± 38
Viraemic pigs (dpc)	7	3/10[a]	3/10[a]	4/10[a,b]	3/10[a]	8/10[b]	0/10
	14	2/10[a]	3/10[a,b]	7/10[b,c]	3/10[a,b]	10/10[c]	0/10
	21	2/10[a]	2/10[a]	5/10[a]	3/10[a]	10/10[b]	0/10
	42	2/10[a]	1/10[a]	3/10[a]	2/10[a]	9/10[b]	0/10
	70	1/10[a]	2/10[a]	2/10[a]	1/10[a]	9/10[b]	0/10
	98	1/10[a]	0/10[a]	1/10[a]	0/10[a]	5/9[b]	0/10
	133	0/10	0/10	0/10	0/10	3/9	0/10
Nasal shedders (dpc)	7	3/10	3/10	4/10	3/10	7/10	0/10
	14	3/10[a]	3/10[a]	6/10[a]	3/10[a]	10/10[b]	0/10
	21	2/10[a]	2/10[a]	4/10[a]	3/10[a]	10/10[b]	0/10
	42	2/10[a]	1/10[a]	2/10[a]	1/10[a]	8/10[b]	0/10
	70	1/10[a]	1/10[a]	2/10[a]	1/10[a]	7/10[b]	0/10
	98	0/10[a]	0/10[a]	1/10[a,b]	0/10[a]	4/9[b,A]	0/10
	133	0/10	0/10	0/10	0/10	1/9	0/10
Histopathological lesion score		0.4 ± 0.5[a,c]	0.3 ± 0.48[a,c]	0.7 ± 0.67[a]	0.9 ± 0.51[a]	1.7 ± 0.70[b]	0.1 ± 0.31[c]
PCV2-antigen score (95% CI)		2.9 ± 4.31[a,c] (0.1–5.9)	3.9 ± 4.19[a,c] (0.8–6.9)	6.2 ± 5.85[a] (2.0–10.4)	7.1 ± 4.65[a] (3.7–10.5)	23.2 ± 6.76[b] (18.4–23.2)	0[c]

Different letters (a, b, and c) indicate statistically significant differences ($P < 0.05$) between groups.
[A] Euthanased due to a leg injury.

Materials and methods

Animal selection and experimental protocol

A total of 60 colostrum-fed, cross-bred piglets from nine PCV2-serongenative gilts were purchased at 14 days old from a porcine reproductive and respiratory syndrome virus (PRRSV)-free commercial farm. Gilts and sows on the farm had never been vaccinated against PCV2, and clinical evidence of PCV2 infection had not been reported. Serum from the piglets was also negative for PCV2 antibodies and virus as indicated by the immunoperoxidase monolayer assay (IPMA) and by real-time PCR (Gagnon et al., 2008; Fort et al., 2009b).

At 3 weeks old, the piglets were assigned to one of six groups (10 pigs/group). For this study we used a randomised, 'blinded', weight- and sex-matched, controlled design. The four commercially available PCV2 vaccines under test were administered according to the manufacturer's instructions with regards to age of vaccination (3 weeks old), and route of injection (IM the right side of neck) as follows: 'Fostera PCV/Suvaxyn PCV2 one dose' (Zoetis) as one 2 mL dose; 'Circovac' (Merial) as one 0.5 mL dose; 'Circoflex' (Boehringer Ingelheim Vetmedica) as one 1 mL dose, and 'Porcilis PCV' (Merck, Sharp and Dohme Animal Health) as one 2 mL dose. Phosphate buffered saline (PBS) was given as a 2 mL dose to the positive and negative control groups.

When the piglets reached 49 days of age (0 days post challenge [dpc]), animals in vaccinated and positive groups were inoculated intranasally with 2 mL (1 mL/nostril) of PCV2b (strain SNUVR000463 [GenBank number KF871068]; 5th passage; 1.0×10^5 50% tissue culture infective dose/mL). Piglets in the negative group were given uninfected cell culture supernatants and served as negative controls.

Each group was housed in separate rooms. Blood samples were collected by jugular venepuncture and nasal samples by sterile swabs (Copan) at −28, 0, 7, 14, 21, 42, 70, 98, and 133 dpc. All pigs were euthanased following tranquilisation by IV azaperone (Stresnil) at 133 dpc (25 weeks of age). All experimental procedures were approved by the Seoul National University Institutional Animal Care and Use Committee (reference SNU-13021911).

Assessment of growth performance

The live weight of each pig was measured at 3 (vaccination), 7 (challenge), 9, 16, and 25 (necropsy) weeks of age. The average daily weight gain (ADWG, g/pig/day) was analysed over four time periods, namely, between 3 and 7, 7 and 9, 9 and 16, and 16 and 25 weeks of age. The ADWG during these various production stages was calculated as the difference between the starting and final weights divided by the duration of the stage. Data from dead pigs were included in the calculation.

Serology and quantification of PCV2 DNA in blood and nasal swabs

Serum was tested using a commercially-available PCV2 IgG ELISA (Synbiotics) and by virus neutralisation (Allan et al., 1994; Pogranichnyy et al., 2000). Samples were considered seropositive where the titre was >550 as per the manufacturer's instructions. DNA was extracted from the serum and nasal samples using the QIAamp DNA mini kit (Qiagen). Extracts were used to quantify PCV2 genomic DNA copy numbers by real-time PCR as previously described (Gagnon et al., 2008).

Enzyme-linked immunospot assay

Numbers of PCV2-specific IFN-γ-SCs were determined in PBMCs at −28, 0, 7, 14, 21, 42, 70, 98, and 133 dpc as previously described (Diaz and Mateu, 2005; Seo et al., 2012). Doses of whole PCV2b virus (as used in the challenge inoculum), at multiples of infection of 0.01, were used to stimulate the PBMCs. Phytohemagglutinin (PHA; 10 μg/mL, Roche Diagnostics) and PBS were used as positive and negative controls, respectively. The concentration of PBMCs was adjusted to contain 10^6 PBMCs/well. The number of IFN-γ-SCs was calculated by the number in the virus-stimulated wells, minus the number in PBS-stimulated wells.

Flow cytometry

PBMCs (10^6 cells/mL) were incubated with R-PE (1 μg/mL)- or FITC (5 μg/mL)-conjugated murine monoclonal antibodies (anti-swine CD3 [R-PE] and CD4 [R-PE and FITC]; SouthernBiotech) for 30 min at 4 °C in the dark and were washed twice. Cells that stained with conjugated antibodies were immediately re-suspended in supplemented RPMI 1640 medium. A 'gate' was set to effect lymphocyte selection using forward/side light scatter information. Background staining was taken into account using the isotype controls conjugated to FITC or PE. Cells were analysed using a FACSCalibur flow cytometer (Becton, Dickinson) as previously described (Sosa et al., 2009).

Histopathology and immunohistochemistry

Morphometric analysis of the histopathological lesion (Fenaux et al., 2002) and PCV2 antigen (Kim et al., 2009) 'scores' in lymph nodes was achieved by examining three superficial inguinal lymph node sections with the examiner 'blinded' to the treatment group. Lesion scores ranged from 0 to 3 as follows: '0' (no lymphoid depletion or granulomatous replacement); '1' (mild lymphoid depletion); '2' (moderate lymphoid depletion), and '3' (severe lymphoid depletion and granulomatous replacement). The number of PCV2-positive cells/unit area (0.25 mm²) of tissue was counted using a NIH Image J 1.45s programme.

Download English Version:

https://daneshyari.com/en/article/2464000

Download Persian Version:

https://daneshyari.com/article/2464000

Daneshyari.com