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Porcine circovirus type 2 (PCV2) vaccination is effective in reducing disease and PCV2 shedding in semen of boars concurrently infected with PCV2 and *Mycoplasma hyopneumoniae*

T. Opriessnig[a,*], D.M. Madson[a], S. Schalk[a], S. Brockmeier[b], H.G. Shen[a], N.M. Beach[c], X.J. Meng[c], R.B. Baker[a], E.L. Zanella[b,d], P.G. Halbur[a]

[a] *Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA*
[b] *Virus and Prion Disease Research Unit, National Animal Disease Center-USDA-Agricultural Research Service, Ames, Iowa, USA*
[c] *Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA*
[d] *Permanent Address: Faculdade de Agronomia e Medicina Veterinaria, Universidade de Passo Fundo, Campus 1, Bairro São José, Passo Fundo, RS, Brazil*

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Abstract

The objectives were to determine whether the amount of porcine circovirus type 2 (PCV2) shed in semen increased in boars experimentally coinfected with *Mycoplasma hyopneumoniae* (MHYO), and whether PCV2 vaccination of boars prior to PCV2 exposure reduced PCV2 viremia and virus shedding in semen. Twelve specific-pathogen-free PCV2- and MHYO-naïve boars were randomly and equally assigned to one of four groups. Six boars were vaccinated against PCV2 (VAC) on Day 0; three PCV2 vaccinated and three non-vaccinated boars were inoculated with MHYO on Day 21, and all boars were challenged with PCV2 on Day 35. The four treatment groups included PCV2-Infected (I), VAC-PCV2-I, MHYO-PCV2-Coinfected (CoI), and VAC-MHYO-PCV2-CoI. Semen, blood swabs, feces, and serum samples were collected weekly until Day 70. All vaccinated boars had seroconverted to PCV2 by Day 35. Between Days 28 and 35, MHYO boars developed moderate respiratory disease, characterized by coughing, respiratory distress, mucopurulent nasal discharge and loss of body condition. One MHYO-PCV2-CoI boar died on Day 50. Boars in the PCV2-I and MHYO-PCV2-CoI groups had significantly higher PCV2 DNA loads in blood swabs than the remaining boars. Moreover, PCV2 vaccination significantly reduced the incidence and amount of PCV2 shedding in semen and feces. In summary, although concurrent MHYO infection did not influence PCV2 shedding patterns, coinfection of boars with PCV2 and MHYO resulted in severe clinical disease and viral shedding was significantly decreased by PCV2 vaccination.

Keywords: *Mycoplasma hyopneumoniae* (MHYO); Porcine circovirus type 2 (PCV2); Semen shedding; PCV2 vaccination; Pig

1. Introduction

Porcine circoviruses (PCV) are small, single-stranded, circular, non-enveloped DNA viruses which belong to the family *Circoviridae* [1]. To date, there are two recognized PCV genotypes referred to as PCV type 1 (PCV1) and PCV type 2 (PCV2) [2,3]. Porcine circovirus type 2 can be further divided into several subtypes, of which PCV2a and PCV2b are prevalent worldwide [4]; PCV2c has been detected in Denmark [5], and PCV2d and PCV2e were detected in China [6].

* Corresponding author: Tel.: +1 515 294 1137; fax: +1 515 294 3564.
E-mail address: tanjaopr@iastate.edu (T. Opriessnig).

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Porcine circovirus type 1 is non-pathogenic [7,8] whereas PCV2 is associated with several disease manifestations, collectively referred to as porcine circovirus associated disease (PCVAD) [9]. Porcine circovirus associated systemic disease or postweaning multisystemic wasting syndrome (PMWS) [10], and PCV2-associated respiratory disease as a part of the porcine respiratory disease complex [11,12], are widely recognized. One less common manifestation of PCVAD is reproductive failure in breeding females, manifested primarily as more mummified fetuses at term [13], usually without clinical signs in the dam [14].

Porcine circovirus type 2 can be transmitted vertically and horizontally. Although the oronasal route is considered the primary route for transmission of PCV2 [15–17], breeding animals could be infected with PCV2 via semen from infected boars. In that regard, PCV2 DNA was shed in semen of both naturally [18–20] and experimentally infected [21,22] boars. Furthermore, PCV2 DNA in boar semen was infectious in a swine bioassay model [23]. However, it was noteworthy that under experimental conditions, the amount of PCV2 shed in semen was low [22] and was not transmitted to naïve breeding animals via AI [23].

In the growing pig model, *Mycoplasma hyopneumoniae* (MHYO) infection potentiated PCV2 replication, PCV2-associated lesions, and clinical disease [24]. Immunomodulation associated with MHYO infection may potentiate the effects of infection with PCV2 [25]. Under field conditions, young boars that enter boar studs are often exposed to infectious agents such as MHYO for the first time, are given multiple adjuvanted vaccines, and are exposed to other stressors (mixing, transportation) thought to enhance PCV2 replication in growing pigs [26].

The main objective of the present study was to determine whether the amount of PCV2 DNA shed in semen increased in boars experimentally coinfected with MHYO and immune-stimulated via administration of an inactivated porcine parvovirus-*Leptospira* spp.-*Erysipelothrix rhusiopathiae* (PLE) vaccine. In addition, the effect of PCV2 vaccination of boars prior to PCV2 exposure on PCV2 viremia and virus shedding in semen was also determined.

2. Materials and methods

2.1. Animals and housing

Twelve 9 month old, specific-pathogen-free (SPF) boars were obtained from an experimental research barrier herd that was continuously maintained for several generations under BSL-3 conditions for more than a decade. This herd was tested regularly and confirmed free of the following pathogens: PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), and MHYO as determined by routine serology and PCR surveillance. After arrival, boars were randomly allocated into six rooms (3 × 4 m; two boars per room) in a BSL-2 facility at the National Animal Disease Center (NADC) in Ames, IA, USA. All rooms were identical in size, had a separate ventilation system, and were equipped with a nipple waterer. Once daily, boars were fed a balanced, pelleted, complete feed ration, free of animal proteins and antibiotics (Nature's Made, Heartland Coop, Cambridge, IA, USA).

Table 1
Experimental design for this study (PCV2, porcine circovirus type 2; PLE, porcine parvovirus-*Leptospira spp.-Erysipelothrix rhusiopathiae*; MHYO, *Mycoplasma hyopneumoniae*).

Group designation	No. boars	Vaccination		Innoculation	
PCV2-I	3		PLE		PCV2
VAC-PCV2-I	3	PCV2	PLE		PCV2
MHYO-PCV2-CoI	3		PLE	MHYO	PCV2
VAC-MHYO-PCV2-CoI	3	PCV2	PLE	MHYO	PCV2

2.2. Experimental design

The experimental design is summarized (Table 1) and the timeline of events is shown (Fig. 1). The 12 boars were randomly and equally assigned to treatment groups and rooms. Each of the six rooms contained one vaccinated (VAC) and one non-vaccinated boar. Three rooms were used for PCV2 infected (PCV2-I) boars, whereas boars in the remaining three rooms were coinfected with PCV2 and MHYO (MHYO-PCV2-CoI) with the following group designations: PCV2-I, VAC-PCV2-I, MHYO-PCV2-CoI, and VAC-MHYO-PCV2-CoI. Porcine circovirus type 2 vaccination was done on Day 0, MHYO inoculation was done on Day 21, PLE vaccination was done on Days 21 and 35, and PCV2 inoculation was done on Day 35. Semen, serum, feces, and blood swabs were collected once weekly from Days 35 to 70, and necropsies were done on Day 70. In addition, six sentinel negative control animals (all females) of similar age, breed and origin as the boars, were kept in three additional rooms in the same building under similar conditions as the boars until Day 70. The experimental protocol was approved by both the NADC and Iowa State University Institutional Animal Care and Use Committees (NADC IACUC number 3972 and Iowa State IACUC number 4-09-6725-S).