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Christian W. Eaton

Hiep Vu

Arabella L. Hodges

Seth P. Harris

Stephen D. Kachman

See next page for additional authors

Authors

Christian W. Eaton, Hiep Vu, Arabella L. Hodges, Seth P. Harris, Stephen D. Kachman, and Daniel C. Ciobanu

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Christian W. Eaton,[1,2] Hiep L. Vu,[1]
Arabella L. Hodges,[1] Seth P. Harris,[3]
Stephen D. Kachman,[4] and Daniel C. Ciobanu [1]

1 Animal Science Department, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
2 School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
3 Veterinary Diagnostic Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
4 Department of Statistics, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

Corresponding author — Daniel C. Ciobanu, dciobanu2@unl.edu

Abstract

Replication of porcine circovirus type 2 (PCV2), an important worldwide swine pathogen, has been demonstrated to be influenced by host genotype. Specifically, a missense DNA polymorphism (*SYNGR2 p.Arg63Cys*) within the *SYNGR2* gene was demonstrated to contribute to variation in PCV2b viral load and subsequent immune response following infection. PCV2 is known to induce immunosuppression leading to an increase in susceptibility to subsequent infections with other viral pathogens

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such as porcine reproductive and respiratory syndrome virus (PRRSV). In order to assess the role of *SYNGR2 p.Arg63Cys* in co-infections, pigs homozygous for the favorable *SYNGR2 p.63Cys* (N = 30) and unfavorable *SYNGR2 p.63Arg* (N = 29) alleles were infected with PCV2b followed a week later by a challenge with PRRSV. A lower PCV2b viremia ($P < 0.001$) and PCV2-specific IgM antibodies ($P < 0.005$) were observed in *SYNGR2 p.63Cys* compared to *SYNGR2 p.63Arg* genotypes. No significant differences in PRRSV viremia and specific IgG antibodies were observed between *SYNGR2* genotypes. Lung histology score, an indicator of disease severity, was lower in the pigs with *SYNGR2 p.63Cys* genotypes ($P < 0.05$). Variation in the lung histology scores within *SYNGR2* genotypes suggests that additional factors, environmental and/or genetic, could be involved in disease severity.

Lay Summary

Porcine circovirus type 2 (PCV2) is an important virus involved in the onset of a group of severe disease symptoms commonly known as porcine circovirus associated diseases (PCVAD). Vaccination options exist for PCV2, though the severity of PCVAD can be influenced by the presence of additional co-infecting pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), for which vaccination is still a challenge. Host genetic resistance is a potential avenue for solving this problem. Previously, a genetic polymorphism in the *SYNGR2* gene was found to be associated with PCV2b viremia and immune response. The aim of this study was to determine the impact of this polymorphism in pigs experimentally co-infected with PCV2b and PRRSV. Pigs were weighed, and blood was collected at various days following infection to measure viremia and antibodies. Histological analysis was performed at the experiment completion to assess disease severity in lungs and lymph nodes. The results showed that variation within the *SYNGR2* gene is involved in PCV2b disease progression including lung histology scores, but no evidence was seen in response to PRRSV infection.

Keywords: host-genotype, PCV2, pig, PRRSV, SYNGR2, virus

Abbreviations: APPV, atypical porcine pestivirus; **PCVAD**, Porcine circovirus associated diseases; **PCV2**, porcine circovirus type 2; **PRRSV**, porcine reproductive and respiratory syndrome virus; **SNP**, single nucleotide polymorphism; **SYNGR2**, synaptogyrin 2; **S/P**, sample/positive control

Introduction

Porcine circovirus type 2 (PCV2) is a small, circular, single- stranded DNA virus belonging to the *Circoviridae* family. PCV2 infection could lead to a systemic group of disease symptoms known as porcine circovirus associated diseases (PCVAD; Segalés, 2012). Field diagnostic

records showed that PCV2 infections are often accompanied by other viral pathogens (Pallarés et al., 2002) such as porcine reproductive and respiratory syndrome virus (PRRSV), a positive-sense, single-stranded RNA virus of the *Arteriviridae* family. PRRSV is considered as one of the most economically important swine pathogens due to respiratory distress and reproductive failure (Rossow, 1998), and lack of robust vaccination options, with an estimated annual impact in the United States of over US $660 million (Holtkamp et al., 2013). Co-infection of PCV2 and PRRSV often results in increased viral replication (Shi et al., 2008; Fan et al., 2013) and disease severity (Drolet et al., 2003; Zeng et al., 2014). Both viral pathogens have been implicated in immunosuppression through inhibition of the host interferon (IFN) response. Specifically, PCV2 hinders IFN-β production through the cGAS signaling pathway, while PRRSV reduces both IFN-α and IFN-β (Calzada-Nova et al., 2011; Wang et al., 2021).

A genome-wide association study (GWAS) followed by in vitro validation approaches led to the discovery of a missense single nucleotide polymorphism (SNP) located in the *SYNGR2* gene, (*SYNGR2 p.Arg63Cys*), as a source of variation in PCV2b viremia and subsequent immune response in experimentally infected pigs (Walker et al., 2018). The cysteine (*Cys*) amino acid allele was associated with lower viremia, specific-IgM and IgG antibodies, and higher average daily gain (ADG). While studied in the context of PCV2 infection, the *SYNGR2 p.Arg63Cys* SNP has yet to be investigated in regards to its effect in co-infection with other swine viruses including PRRSV. We hypothesized that the reduction in PCV2b viremia associated with the favorable *SYNGR2* genotype (*Cys/Cys*) would result in reduced PRRSV viral load following co-infection. In this study, the role of this polymorphism was assessed in pigs homozygous for alternate *SYNGR2 p.Arg63Cys* alleles infected with PCV2b followed by co-infection with PRRSV a week later.

Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska-Lincoln.

Experimental design: animals, diet, and housing

Experimental challenge was conducted using Landrace (dam) × Yorkshire (sire) barrows (*N* = 59), selected based on multiple criteria including, 1) *SYNGR2 p.Arg63Cys* genotype, *Arg/ Arg* (*N* = 29) and *Cys/ Cys* (*N* = 30), 2) absence of common viral pathogens, such as PCV2, PCV3, PRRSV, or atypical porcine pestivirus (APPV), and 3) limited levels of PCV2-specific IgG maternal antibody (S/(*P* × 0.3) < 1). As industry standards, both sires and dams were vaccinated for PCV2. The source farm is using industry vaccination programs for multiple pathogens and also routine testing for PRRSV. The experimental piglets were not vaccinated for PCV2 or PRRSV. The animals were born within an interval of 3 d and weaned at an age of 18 to 20 d.

Following weaning, the experimental animals were transported from the source farm to the UNL Life Science Annex and distributed in four BSL2 rooms, based on *SYNGR2* genotype, litter information and passive PCV2-specific IgG decay. Animals were derived from 19 litters and pooled semen. The number of piglets used from each litter ranged from 1 to 7 with an average of 3.1 pigs per litter. Since the decay of maternal antibodies was different across animals (litters), the piglets were distributed in two batches, with two rooms per batch. There was a difference of 7 d in the challenge time between batches. At the time of challenge, all experimental pigs had low levels of passive (maternal) antibodies (S/(P × 0.3) < 1) that would permit successful infection, as previously demonstrated (Engle et al., 2014). Prior to experimental challenge, all the animals tested negative for PCV2b and PRRSV using qPCR assays as described below. All animals were allowed ad libitum access to a balanced diet which met or exceeded nutritional requirements (NRC 2012).

The vast majority of the litters had piglets allocated to more than one room (89.5%). Specifically, 12 of the litters had piglets allocated to two rooms (63.2%), four to three rooms (21%) and one to all four rooms (5.3%). Six of the 19 litters (21%) included piglets of both genotypes, generating 54% of the experimental animals used in the study (*N* = 32). In the experimental design, the plan was to allocate an equal number of genotypes per batch and experimental room, and to a certain extent, we were able to do that (**Table 1**).

Table 1. Distribution of the SYNGR2 genotypes of experimental piglets across batches and rooms

Batch	Room	*SYNGR2* genotypes	
		Arg/Arg	Cys/Cys
B1	R1	8	8
	R2	8	9
B2	R3	7	6
	R4	6	7

PCV2b and PRRSV experimental challenge, data, and sample collection

Animals were challenged with PCV2b (strain UNL2014001) following weaning and passive PCV2 IgG antibody decay, *via* intramuscular injection (1 ml) and intranasal spray (1 ml in both nostrils) with a titer of $10^{4.0}$ TCID50/ml as previously described (McKnite et al., 2014). After 7 d, the animals were subsequently co-infected with PRRSV (strain FL12) *via* intramuscular injection (2 ml) with a titer of $10^{5.0}$ TCID50/2 ml. The virulence of these strains has been previously described (Truong et al., 2004; Engle et al., 2014; McKnite et al., 2014).

Animal weights were recorded at 0, 7, and 17 d, and blood was collected at 0, 4, 7, 11, 14, and 17 d after PCV2b challenge. Serum was obtained through centrifugation of whole blood samples at 2,400 × *g* for 30 min at 4 °C. Viral DNA was isolated from serum for the detection of PCV2b using the QIAamp 96 DNA Blood Kit (Qiagen) according to the manufacturer's protocol with minor modifications for enhanced binding of viral nucleic acids. Viral RNA was isolated from serum for the detection of PRRSV utilizing the MagMAX-96 Viral RNA Isolation kit (Applied Biosystems) and MagMAX Express Magnetic Particle Processor (Applied Biosystems) according to the manufacturer's protocol. PRRSV cDNA was obtained from isolated RNA using the First-Strand cDNA Synthesis Kit (Cytiva) according to the manufacturer's protocol.

At the end of the experiment, 54 piglets, equally representing both *SYNGR2* homozygote genotypes, (*Arg/Cys* 27/27) were euthanized for necropsy. The right cranial lung lobe, right middle lobe, right caudal lobe, accessory lobe of right lung, cranial aspect of the left cranial lobe, caudal aspect of the left cranial lobe, and left caudal lobe were

grossly scored for discoloration and consolidation. The scores represent the percentage, by volume, of each lung lobe grossly affected following infection. Three samples of lung from the same region of each pig and a tracheobronchiolar lymph node were fixed in 10% neutral buffered formalin and routinely processed overnight on a Tissue-Tek VIP 5 Tissue Processor. The resultant tissue pieces were embedded in paraffin blocks and cut to 5 μm thickness using a Leica RM2255 microtome and stained with hematoxylin and eosin on a Leica ST5020 H&E stainer, and coverslipped with a Leica CV5030 coverslipper.

Lung and tracheobronchiolar lymph node lesions were histologically graded by a board certified veterinary anatomic pathologist using a previously described grading scale (Krakowka et al., 2005). Lung scoring was done using a subjective grading scale from 0 to 4 to assess the severity of interstitial inflammation in the lung sections. A grade of 0 was assigned to normal lungs, a grade of 1 was assigned to lungs with minimal interstitial pneumonia, a grade 2 was assigned to lungs with moderate interstitial pneumonia in multifocal regions, a grade 3 was assigned to lungs with severe interstitial pneumonia that had a multifocal to coalescing pattern, and a grade 4 was assigned to lungs with severe interstitial pneumonia that diffusely affected the tissue. Tracheobronchiolar lymph node sections were scored with a grading scale from 0 to 4 in severity as described in Krakowka et al. (2005). A grade of 0 was assigned to normal lymph nodes, a grade of 1 was assigned to minimal lesions, a grade 2 was assigned to moderate lesions, a grade 3 was assigned to severe lesions, and a grade 4 was assigned to very severe lesions that were diffuse. Lesions in the lymph node were assessed for lymphoid depletion, lymphoid hyperplasia, germinal center development, reticuloendothelial hyperplasia, and histiocytic and giant cell infiltration.

Serum analysis

PCV2b viremia was quantified using a TaqMan-based qPCR assay (forward: 5′ – TGG CCC GCA GTA TTC TGA TT – 3′; reverse: 5′ – CAG CTG GGA CAG CAG TTG AG – 3´; probe: 5´– FAM – CCA GCA ATC/Zen/AGA CCC CGT TGG AAT G – IABkFQ – 3′). PRRSV viremia was obtained using TaqMan-based RT-qPCR (forward: 5′ – GCA TGG TTC TCG CCA ATT AAA – 3′; reverse: 5′ – ATG TGT GGT GAA TGG CAC TG

– 3′; probe: 5′ – FAM – TCA CCT ATT/Zen/ CAA TTA GGG CGA CCG – 3IABkFQ – 3′). The TaqMan Universal Master Mix (Life Technologies) and a CFX384 Real-Time PCR (BioRad) instrument were used to perform qPCR amplifications. Viremia was measured as an estimate of viral copies/mL by fitting to a linear model calculated as the log-transformed cycle threshold value plotted against the dsDNA input of serially diluted virus-specific controls. The controls were generated using PCR amplification of template viral DNA (PCV2) or cDNA (PRRSV) purified by QIAquick Gel Extraction Kit (Qiagen) according to manufacturer's protocol. The dsDNA concentration of the amplicon used as control was quantified using a Qubit fluorometer (Invitrogen).

PRRSV-specific IgG and PCV2-specific IgG and IgM antibody levels were measured at each blood collection following challenge using ELISA. Specifically, INgezim Circovirus ELISA kit (Ingenasa) was used to quantify IgM and IgG PCV2-specific antibodies, expressed as a measure of the sample to positive control (S/P) ratio, according to the manufacturer's protocols. IDEXX PRRS X3 ELISA kit was used to determine IgG PRRSV-specific antibody, also expressed as a S/P ratio. The PCV2-specific antibody S/P ratio was normalized, as previously described (Engle et al., 2014), as follows: for the IgM antibodies, the raw read for each sample was divided by the raw read from the positive control, provided in the kit, multiplied by 0.4 (S/(P × 0.4)); for the IgG antibodies, the raw read for each sample was divided by the raw read from the positive control multiplied by 0.3 (S/(P × 0.3)). A sample was determined to be actively infected (IgM) or previously exposed to PCV2 (IgG) if the normalized S/P ratio was ≥1. The PRRSV-specific IgG antibody S/P ratio was calculated by dividing the raw read of each sample by the positive control raw read result, with a ratio ≥ 0.4 indicating pigs were previously exposed to PRRSV.

Genotyping

DNA was isolated from ear clips using the DNeasy blood and tissue kit (Qiagen). The experimental pigs were genotyped for *SYNGR2 p.Arg63Cys* using KASPar (LGC), a genotyping detection assay based on allelic-specific amplification and fluorescent resonance energy transfer. There were 223 animals from the source farm genotyped for *SYNGR2 p.Arg63Cys* in order to select candidate piglets for the

experiment. Of these 223 animals genotyped, 44 were homozygous for the arginine (*Arg*) allele, 48 were homozygous for the *Cys* allele, and 131 were heterozygous. Additional genotyping was performed for an intronic SNP (rs340943904) located in the *GBP5* gene, previously associated with PRRSV viremia (Koltes et al., 2015), using PCR-based restriction fragment length polymorphism (RFLP) analysis. Specifically, following PCR amplification (forward: 5′– GAG TGG GAG GGA GAA GGA T – 3′; reverse: 5′– GGC TCT CTG TTG CAA TAC CT – 3′), the DNA was digested with *AluI* (NEB) and separated by gel electrophoresis to differentiate the genotypes.

Statistical analysis

Statistical relationship between *SYNGR2* genotypes and phenotypic measurements were assessed using a linear mixed model including host genotype, batch and experimental room nested within batch as fixed effects, litter nested within experimental room as random effects, and PCV2 IgG antibody level at day 0 (passive antibodies) as a covariate. The additive effect was estimated as a half of the difference between the effect of *Arg/Arg* and *Cys/Cys* genotypes. Relationship between phenotypic measurements were estimated using Pearson's correlation co-efficient.

Results

Histological examinations provide consistent detection of disease severity

Disease resilience at the farm level could be defined as a relationship between measures of disease progression (e.g. viremia) and one of the most economically important traits, ADG during infections. In this study, a negative relationship was detected between PRRSV viremia and ADG after 3 d following PRRSV challenge ($r < -0.49$, $P \leq 0.0002$). While the direction of the relationship between PCV2 viremia and growth confirmed our expectation and previous reports (Engle et al., 2014), the correlations between overall ADG and PCV2b viremia at day 11 ($r = -0.10$) and 14 ($r = -0.13$) following challenge were not

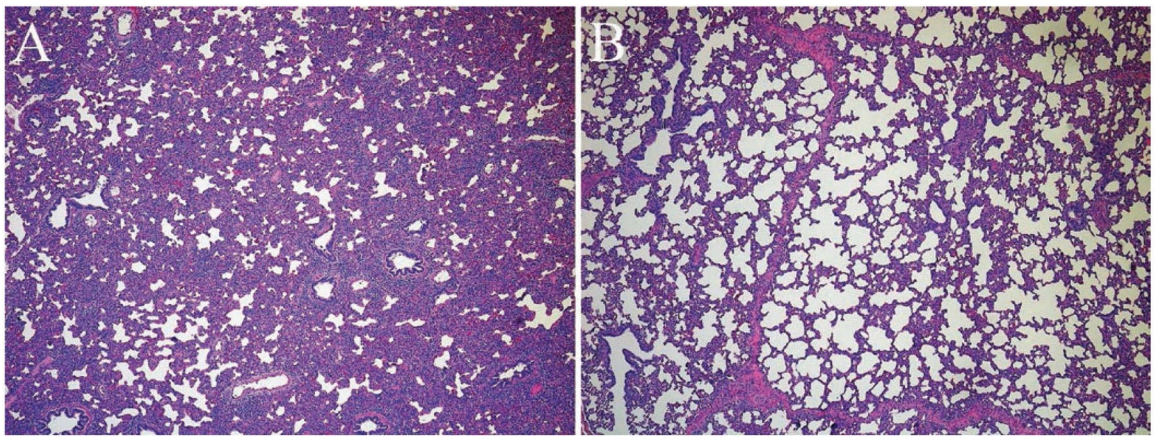


Figure 1. Severe interstitial pneumonia that diffusely affected the tissue observed in lung from a pig homozygous for the *SYNGR2 p.63Arg* allele (score 4) (A). Moderate interstitial pneumonia in multifocal regions observed in lung tissue from a pig homozygous for the *SYNGR2 p.63Cys* allele (score 2) (B).

significant *(P* > 0.05). A positive relationship was observed between PCV2b viremia on day 7 and PRRSV viremia on day 17 post challenge with PCV2b (r = 0.44, *P* < 0.005), indicating the potential early role of PCV2 in PRRSV replication.

While viremia and ADG are indicators of infection progress, histological examinations of the affected tissues represent robust measures of disease severity. For example, histological evaluation of the experimental samples was more consistent in detecting interstitial pneumonia than gross examination (**Figure 1** A, B). Postmortem collapse of air spaces and congestion rapidly alters the appearance of the tissues grossly and as a result not all the pigs reached the threshold of gross detection of interstitial pneumonia following necropsy. In contrast, histological examination demonstrated the presence of moderate to severe pneumonia in all of the piglets. Average lung histology score was elevated in the majority of the pigs, with the exception of five individuals that maintained a lower average score (≤3). The vast majority of the samples (91%) had a high histology score (>3.3; **Figure 2**), with a range of 3.33 to 4. There was almost no variation in the tracheobronchiolar lymph node histology score. Specifically, all but one sample were assigned to moderate lymph node lesions grade (grade 2; 98.2%).

Figure 2. Average lung histology scores for each pig, by their *SYNGR2 p.Arg63Cys* genotype.

SYNGR2 host genotypes explained variation in PCV2 viremia and antibody response

A significant difference in PCV2b viremia was observed between animals with alternate *SYNGR2* homozygous genotypes beginning 11 d post PCV2b challenge, a trend that continued throughout the rest of the challenge period (17 d post PCV2b infection; **Figure 3**; **Table 2**). Specifically, piglets with the *Cys/Cys* genotype exhibited lower viremia than those with the *Arg/Arg* genotype ($P < 0.001$), which is consistent with our previous reports (Walker et al., 2018). More specifically, an increase in PCV2b viremia was delayed in animals with the *Cys/Cys* genotype compared to *Arg/Arg* genotype, with a significant difference in slope observed from days 7 to 11 post challenge ($P < 0.01$).

PCV2-specific IgM is the primary antibody and an indicator of active PCV2 infection. Following challenge, the PCV2-specific IgM antibody level increased over the course of the experiment in all animals, regardless of *SYNGR2* genotype. However, the increase was

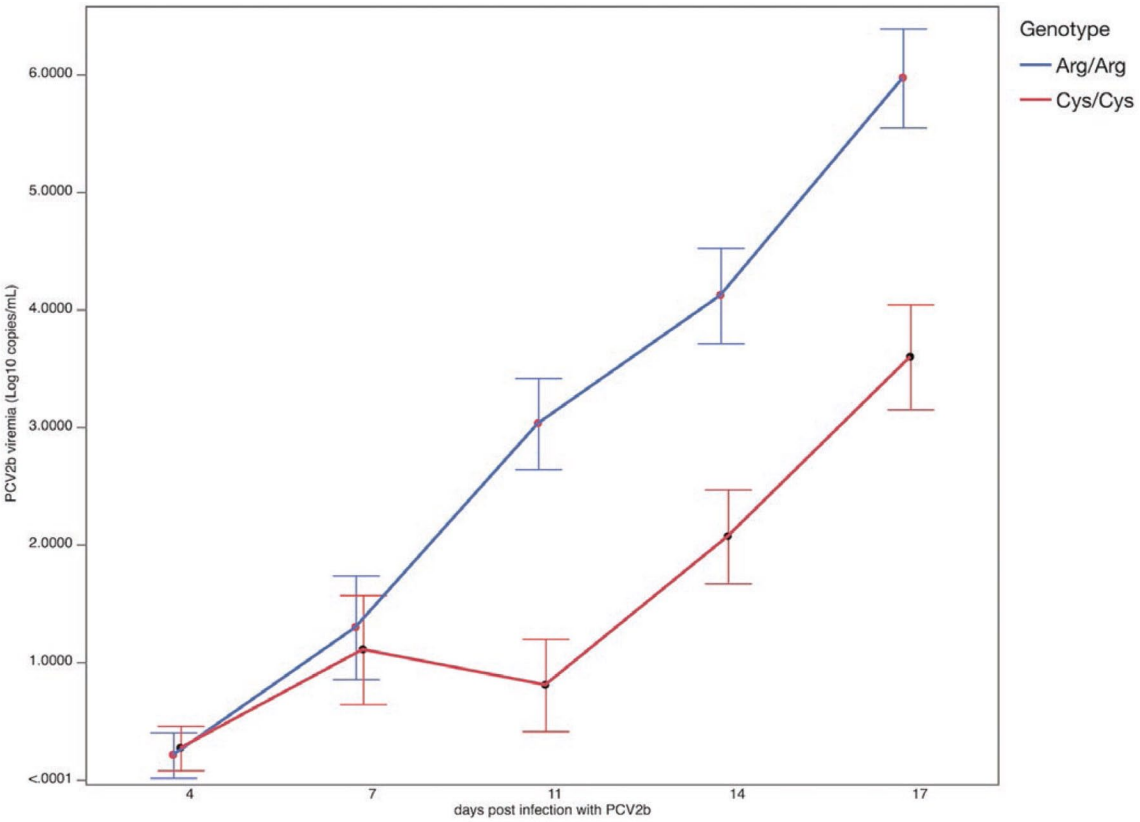


Figure 3. PCV2b viremia for *SYNGR2 p.Arg63Cys* genotypes across time points following experimental infection. Least square means with standard error shown in brackets.

more pronounced in animals with the *Arg/Arg* genotype, with significantly higher levels of IgM starting at 14 d post PCV2b challenge, compared to the *Cys/Cys* genotype ($P < 0.005$; Table 2; **Figure 4**).

PCV2-specific IgG antibody is an indicator of previous exposure to PCV2, PCV2 vaccination or passive immunity acquired *via* colostrum. As in our previous reports (Engle et al., 2014), average IgG levels, an indicator of active immunity following infection, started to increase at day 14. As a result of a later seroconversion for the PCV2-specific IgG antibodies, no significant difference in PCV2-specific IgG antibody response was observed between *SYNGR2* genotypes across days, but a numerical difference was observed on day 17 ($P = 0.10$; **Figure 5**) with the *Cys/Cys* genotype expressing a lower level of IgG (Table 2).

Table 2. Least square means (LSM) and additive effect of the *SYNGR2 p.Arg63Cys* genotypes for measures of PCV2 and PRRSV infection, immune response, lung histology scores and ADG

Phenotype	*dpi*	*LSM(SE)*		*Units*	*Additive Effect*	*P value*
		Cys/Cys	*Arg/Arg*			
PCV2b viremia	4	0.281(0.186)	0.208(0.191)	Log_{10} copies/mL	−0.0363	0.7853
	7	1.112(0.464)	1.302(0.441)		0.0948	0.7613
	11	0.81(0.393)	3.036(0.386)		1.1127	0.0001
	14	2.075(0.399)	4.125(0.406)		1.0253	0.0007
	17	3.602(0.448)	5.976(0.42)		1.1871	0.0003
PCV2 IgM antibodies	0	0.393(0.034)	0.407(0.031)	S/P ratio	0.0069	0.7067
	4	0.461(0.027)	0.453(0.027)		−0.0040	0.8349
	7	0.545(0.054)	0.574(0.054)		0.0147	0.6982
	11	0.684(0.089)	0.822(0.09)		0.0689	0.2784
	14	0.907(0.094)	1.323(0.099)		0.2082	0.0034
	17	0.982(0.121)	1.547(0.124)		0.2825	0.0020
PCV2 IgG antibodies	0	0.566(0.03)	0.57(0.027)	S/P ratio	0.0021	0.8980
	4	0.481(0.021)	0.472(0.019)		−0.0044	0.7212
	7	0.481(0.021)	0.485(0.018)		0.0019	0.8844
	11	0.404(0.024)	0.428(0.024)		0.0118	0.4905
	14	0.431(0.054)	0.51(0.056)		0.0396	0.3140
	17	0.412(0.082)	0.606(0.083)		0.0968	0.1025
PRRSV viremia	11	8.711(0.083)	8.555(0.079)	Log_{10} copies/mL	−0.0779	0.1773
	14	8.894(0.07)	8.885(0.072)		−0.0044	0.9304
	17	8.71(0.075)	8.605(0.071)		−0.0522	0.3092
PRRSV IgG antibodies	7	−0.002(0.005)	−0.004(0.004)	S/P ratio	−0.0009	0.7541
	11	−0.003(0.002)	−0.007(0.002)		−0.0020	0.1483
	14	0.78(0.062)	0.736(0.059)		−0.0219	0.5938
	17	1.873(0.063)	1.937(0.056)		0.0323	0.3906
ADG	0-7	0.768(0.064)	0.804(0.062)	lb/day	0.0179	0.6853
	7-17	1.046(0.069)	1.082(0.071)		0.0176	0.7225
	0-17	0.927(0.044)	0.978(0.045)		0.0253	0.4232
Avg. lung histology score		3.498(0.082)	3.752(0.084)		0.1267	0.0351

SYNGR2 host genotypes did not explain variation in PRRSV viremia and antibody response

PRRSV genomic RNA was detected on 4 d, reaching a peak on 7 d following PRRSV challenge. Contrary to our hypothesis, no significant differences in PRRSV viremia were detected between *SYNGR2* genotypes on any of the days following infection *(P* ≥ 0.177; Table 2; **Figure 6**). An increase in PRRSV-specific IgG antibody was evident 7 d after PRRSV challenge. No significant difference in IgG levels between *SYNGR2* genotypes was observed, but piglets with the *Cys/ Cys* genotype exhibited a numerical lower level of antibody (*P* ≥ 0.148; Table 2; **Figure 7**).

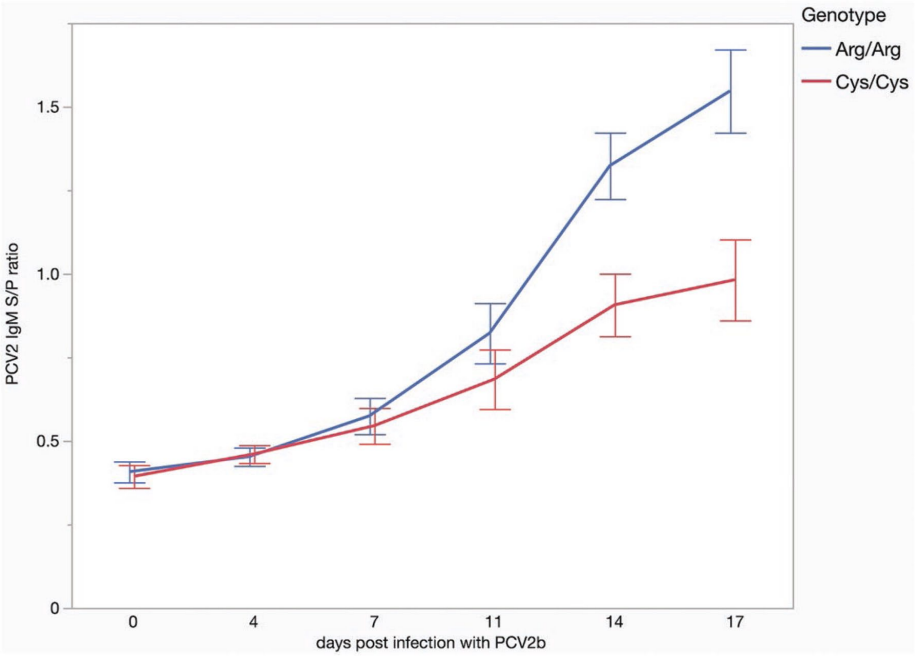


Figure 4. PCV2-specific IgM antibody for *SYNGR2 p.Arg63Cys* genotypes across time points following experimental infection. Least square means with standard error shown in brackets.

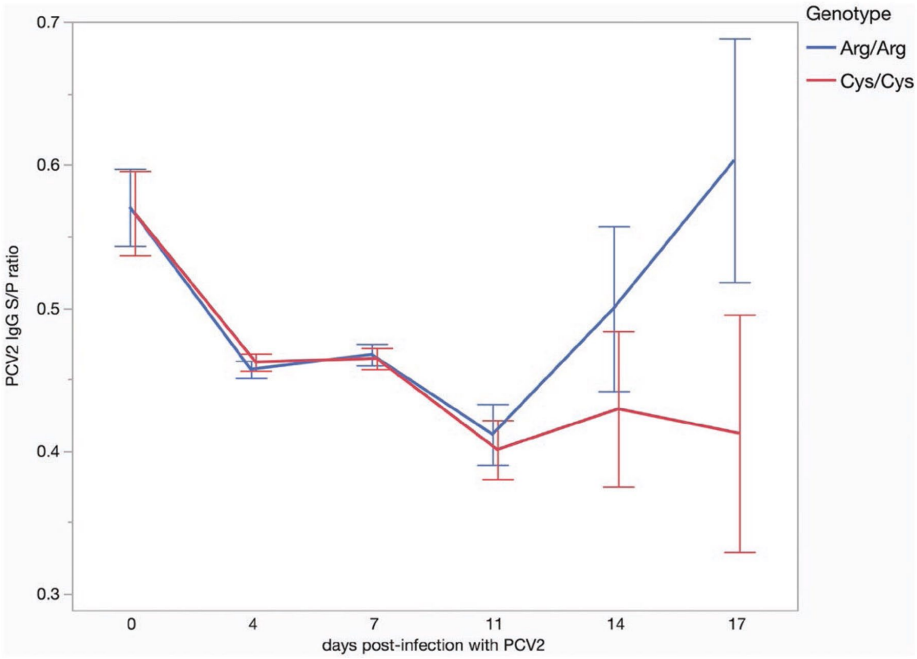


Figure 5. PCV2-specific IgG antibody for *SYNGR2 p.Arg63Cys* genotypes across time points following experimental infection. Least square means with standard error shown in brackets.

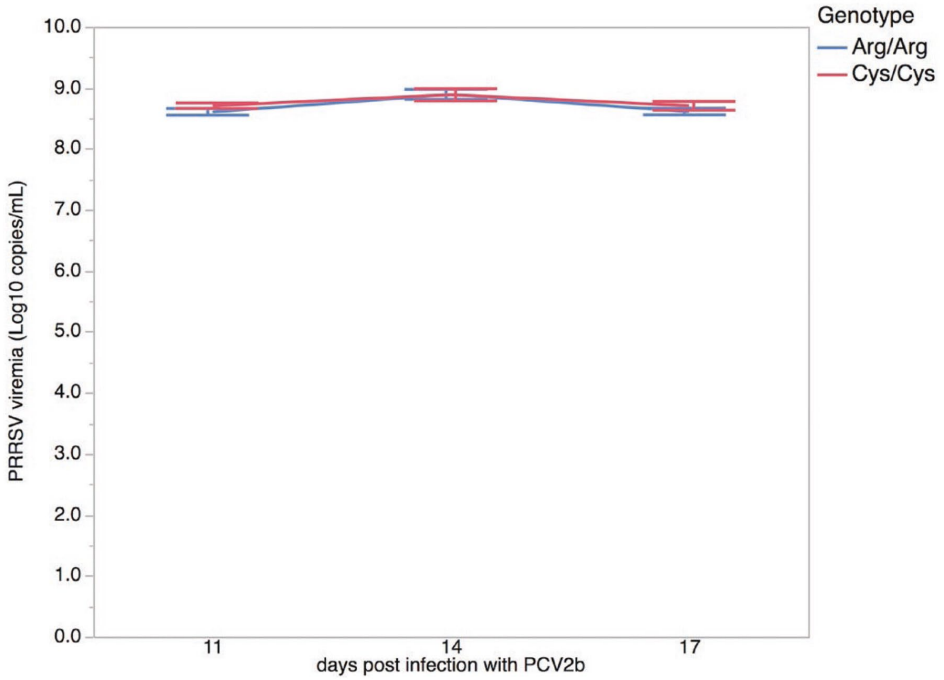


Figure 6. PRRSV viremia for *SYNGR2 p.Arg63Cys* genotypes across time points following experimental infection. Least square means with standard error shown in brackets.

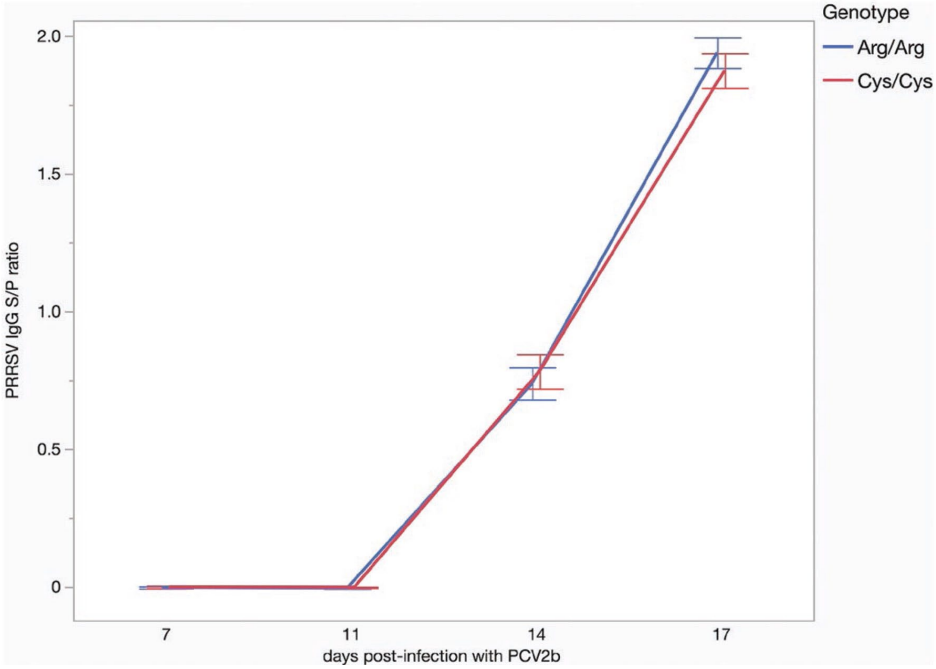
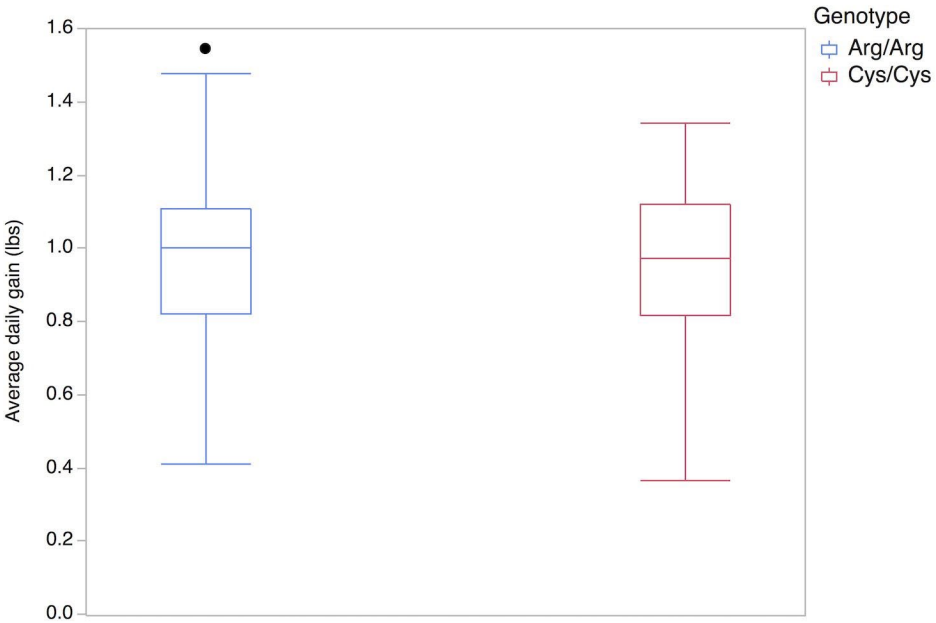


Figure 7. PRRSV-specific IgG antibodies for *SYNGR2 p.Arg63Cys* genotypes across time points following experimental infection. Least square means with standard error shown in brackets.

Host genotype could play a role in the severity of PCV2 and PRRSV disease outcome

Histological analysis was performed to test if some of the variation in severity of lung and tracheobronchiolar lymph node tissue damage attributed to PCV2 and PRRSV co-infection could be explained by the host genotypes. Despite a limited variation in lung tissue damage, a lower average lung histology score was observed in individuals with the *Cys/Cys* genotype, indicative of decreased disease severity ($P < 0.05$; Table 2; Figure 4). This difference was potentially driven by a cluster of five individuals, all with the *Cys/Cys* genotype, that exhibited the lowest average lung histology scores (≤3; Figure 2). In addition, no difference in ADG was detected, between the *SYNGR2* genotypes during early, late or the entire challenge ($P > 0.43$; Table 2; Supplementary Figure S1).



Supplementary Figure S1

Discussion

Previously, a GWAS followed by in vitro validation demonstrated the direct effect of the host *SYNGR2* gene on PCV2b infection (Walker et al., 2018). A missense polymorphism in *SYNGR2* (*SYNGR2 p.Arg63Cys*) was associated *in vivo* with variation in PCV2b viremia and subsequent PCV2-specific immune response. Consistent with our previous reports, in this study, we validated the role of *SYNGR2 p.Arg63Cys* in PCV2 viremia and antibodies. While the two previously reported data sets (Walker et al, 2018; N_D = 974 and N_V = 69) are not directly comparable (all three genotypes present, 28 d challenge, etc.), we found the same direction and magnitude of the effect of the *SYNGR2 p.Arg63Cys*. With an increase in the number of *SYNGR2 p.63Cys* favorable alleles, there is a subsequent reduction in PCV2b viremia and antibodies. Specifically, at 14 d following challenge with PCV2b, a common time point following PCV2 challenge across data sets, the effect of the *SYNGR2 p.Arg63Cys* polymorphism in PCV2 viremia was similar in this study (N = 59, −1.05 $\log_{10}$ copies/ ml) compared to the diversity (N_D = 440; −0.69) and the validation data sets (N_V = 69; −0.59) used in Walker et al. (2018).

The diversity PCV2 infection data set (N_D = 974) consists of experimental pigs representing 14 genetic lines and seven genetic programs. Similar results were observed for PCV2-specific antibody response. On 14 d following challenge, the additive effect of the *SYNGR2 p.Arg63Cys* polymorphism in IgM was similar in this study (N = 59; −0.21) compared to the previously reported results in the diversity (N_D = 440; −0.19) and the validation data sets (N_V = 69; −0.15) (Walker et al., 2018). Both the current and previous data sets used the same PCV2b strain, similar titers and experimental challenge. The consistency of the genotype effect, despite smaller data sets being investigated, is a result of the causative/functional role of this polymorphism on PCV2b infection.

The lack of correlation seen between PCV2b viremia and ADG in this study is potentially due to sample size (N = 59). An important negative relationship (r = −0.35, P < 0.001) was observed previously between PCV2 viral load and ADG in a larger, highly diverse, experimental challenge with PCV2b (N_D = 974; Engle et al., 2014). We demonstrated that the *SYNGR2 p.63Cys* allele was associated with higher

ADG following PCV2b infection ($P < 0.001$; Walker et al., 2018). The lack of the SYNGR2 genotype effect on ADG and limited effect on lung pathology during PCV2/PRRSV co-infection could be also due to PRRSV. The pigs with the *Cys/ Cys* genotype had lower PCV2 viral load and therefore were expected to experience less severe disease, specifically, lower lung score that could lead to high ADG. However, as these pigs were also infected with PRRSV and *SYNGR2* genotype does not appear to influence its replication, PRRSV is expected to cause lung damage and affect growth in all pigs, regardless of genotype, "erasing" part of the effect of host-genotype on PCV2-triggered lung disease.

Although no difference was detected between *SYNGR2* genotypes for PRRSV viremia or PRRSV-specific IgG antibodies, a different outcome may have resulted if the PRRSV challenge was initiated at a later time point, when the difference in PCV2b viremia between host genotypes was larger, such as at 11 d following challenge with PCV2b ($P < 0.001$). This could have potentially led to a difference in PRRSV replication between *SYNGR2* genotypes due to the expected synergistic interaction between PCV2b and PRRSV. Alternatively, other studies, which utilized a simultaneous co-infection of PCV2b and PRRSV, have suggested that co-infection with PCV2 does not always lead to a subsequent increase in PRRSV viral load (Allan et al., 2000; Tu et al., 2015). The timing of the experimental challenge for PCV2b/PRRSV co-infection is therefore of interest as both a simultaneous challenge, and a challenge first with PRRSV followed by PCV2b have not yet been performed to evaluate the role of host *SYNGR2* genotype in the co-infection of these two viruses.

The potential role of other QTLs that solely influence PRRSV viremia were also considered. For example, an intronic SNP in the *GBP5* gene (rs340943904) was implicated in host-genetics response to PRRSV infection (Koltes et al., 2015). This polymorphism was found monomorphic in our data set, all piglets being homozygotes for the allele associated with a higher PRRSV viremia (Koltes et al., 2015). A previous report from the analysis of a large data set of pigs simultaneously co-infected with PCV2b and PRRSV, following vaccination for PRRSV, suggested that *GBP5* genotype may additionally have an effect on PCV2b viral load (Dunkelberger et al., 2017). More recently, polymorphisms in genes with known biological impact on PRRSV infection (e.g., *CD163* and *CD169)* were evaluated for their effect and

interactions with *GBP5* genotype in PRRSV-only and simultaneous PCV2/PRRSV co-infections (Dong et al., 2021). The findings of these studies, and of the current study, underlined the polygenic nature of disease outcome in PCV2b/PRRSV infections, and additional research needed to characterize the complexity of genetic susceptibility in infectious disease in pigs.

Conclusion

Our previous study demonstrated a clear relationship between host-genetics and the severity of PCV2 infection in pigs (Walker et al., 2018). Specifically, the *SYNGR2 p.Arg- 63Cys* polymorphism explained part of the variation in PCV2 replication, with the *Cys* allele associated with lower viremia and PCV2-specific IgM following infection. Using homozygote pigs for the *SYNGR2 p.Arg63Cys* polymorphism, the current study validated our previous reports and, in addition, provided a possible indication of the role of this polymorphism in disease severity based on histological scores of the lung. The potential combined role of *SYNGR2* host-genotype and PCV2 immunosuppression in PRRSV co-infection was not observed since no difference in PRRSV viremia and antibody levels were detected between *SYNGR2 p.Arg63Cys* genotypes. Further research of the mechanistic role of *SYNGR2 p.Arg63Cys* or other host-genetic factors in viral infection could provide the knowledge to understand differences in disease severity that could ultimately lead to molecular solutions to improve animal health and welfare.

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Conflict of Interest Statement The authors declare no real or perceived conflicts of interest.

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