

# Quantitative Effect of Porcine Reproductive Respiratory Syndrome Virus on Pig Growth and Immune Response

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### Summary and Implications

Forty-eight pigs from a herd naïve for porcine reproductive respiratory syndrome virus (PRRS) were weaned, placed in isolation chambers, and oral-nasally inoculated with 2 ml of  $10^4$  JA142 PRRS virus. For each pig, body weight, feed intake, and serum concentration of PRRS virus titers, gamma-interferon ( $\gamma$ -IFN), and alpha-1-glycoprotein (AGP) were determined every 4 days for 24 days post-inoculation to determine the effect of PRRS exposure on growth and immune response in pigs and to quantify the relationship between serum virus concentration and pig growth. Serum virus titers and  $\gamma$ -IFN, both peaked at 4 days post-inoculation, and then declined steadily throughout the 24 day study. As expected, serum AGP responses were delayed with peak concentrations occurring 12 days post-inoculation. Body weight gains and feed intakes of individual pigs were quantitatively related to the animal's serum concentration of virus titers and to a lesser degree to serum concentration of  $\gamma$ -IFN and AGP. Specifically, each additional 10-fold of serum virus titer was associated with a mean reduction of .018 kg in daily pig gain and .028 kg in daily pig feed consumption. These data indicate that the magnitude of biological responses that occur in pigs infected with PRRS is directly related to the animal's serum virus concentration.

### Introduction

Porcine reproductive and respiratory syndrome virus (PRRS) is a widespread viral disease that has a great impact on the swine industry. PRRS has been shown to infect macrophages within the pig's immune system. These infected macrophages become the host cell for the virus while it replicates. The virus eventually ruptures the cell and is released into the body. In young pigs, symptoms of an infection include fever, anorexia, vomiting, and coughing (1).

During a viral challenge, the pigs immune system becomes activated. One of the initial immune responses that occurs is the release of cytokines. These cytokines stimulate the proliferation of immune cells and the production of

antibodies. The chronic release of proinflammatory cytokines also results in the reduction of body growth, particularly muscle growth, and the efficiency of feed utilization.

Although PRRS has been shown to decrease growth and feed intake, it has not been determined if the magnitude of depression is quantitatively related to serum virus concentration present in the animal. Therefore, the objective of this study was to determine if the magnitude of the biological responses of the pig to PRRS is quantitatively related to levels of PRRS exposure as measured by serum concentration of PRRS titer.

### Materials and Methods

**Animals.** Forty-eight pigs from a high lean strain were obtained from a herd naïve (noninfected, nonvaccinated) for PRRS. Pigs were weaned at  $11 \pm 2$  days of age, penned individually in 2 x 4 foot slatted floor pens, and allowed to consume feed and water ad libitum. Pigs were reared in the disease isolation chamber at the National Animal Disease Center, Ames, IA, to minimize the animal's exposure to other antigens. A thermal climate of 82° to 75°C was maintained. Pigs were self-fed a diet that exceeded the estimated nutrient requirements of high lean pigs fed from 5 to 18 kg (2). Pigs received no therapeutic treatment during the study.

Eighteen days following weaning, the pigs were oral-nasally inoculated with 2 ml of  $10^4$  PRRS virus strain JA142 (courtesy of William Mengeling, NADC, Ames, IA). Blood was collected every 4 days for 24 days to determine serum concentrations of virus titers, gamma-interferon ( $\gamma$ -IFN), and alpha-1-glycoprotein (AGP). Feed intake and gain were also measured every 4 days for 24 days to determine daily feed intakes and daily gains for each period post-inoculation.

**Blood samples.** Blood also was collected on day 0 (prior to inoculation) and day 24 of the study for determination of titers for PRRS, transmissible gastroenteritis, swine influenza virus, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae*. The samples were analyzed by the Iowa State University Veterinary Diagnostic Laboratory. The tests performed were an ELISA, serum neutralization, hemagglutination inhibition, ELISA, and complement fixation respectively. These samples were taken to verify the animals did not have passive or active titers for PRRS or the other prevalent pig antigens monitored.

**Virus titer.** MARC 145 cells were grown in culture media for 7 days. Cells were then removed from culture flasks and diluted into 500 ml of media. Cell media (150  $\mu$ l)

was placed in each well of a 96-well plate. The cells were then allowed an additional 3 days for multiplication. Eight 1:10 serial dilutions of each serum sample were made, and then 50  $\mu$ l of the diluted sera was added to the plates. Each sample was ran in 11 replications for each dilution. The virus was then allowed 5 days for viral replication. The wells were evaluated based on the cytotoxic pathogenic effect (CPE) of the virus.

**Gamma-interferon.** Gamma interferon levels were assayed using a modified cell bioassay (3). Briefly, two-fold serial dilutions of sera were prepared in triplicate with Eagle Minimum Essential Medium (MEM) in a 96-well plate. Dilute sample (100  $\mu$ l/well) and 50  $\mu$ l of Madin Darbey bovine kidney (MDBK) cells were incubated for 24 hours. Fifty microliters of vesicular stomatitis virus (VSV) was added to each well. Plates were incubated another 24 hours before addition of 100  $\mu$ l of MTT per well. Plates were then incubated for 2 hours, centrifuged, emptied by inversion, washed two times with PBS before the addition of 150  $\mu$ l isopropanol to solubilize formazan crystals. The amount of formazan produced by the MDBK cells was directly proportional to viable cell number. Because  $\gamma$ -IFN protects the MDBK cells from killing by VSV, absorbance at 570 nm due to formazan production was used to measure the amount of  $\gamma$ -IFN in each serum sample. Concentrations of  $\gamma$ -IFN are reported as a percentage of cell protection.

**Alpha-1-glycoprotein.** Serum AGP was analyzed by radial immunodiffusion (4).

**Data analysis.** Data were analyzed using the GLM and regression procedures of SAS (1998). Response over time were analyzed as a repeated measure. The pig was considered the experimental unit.

## Results and Discussion

**Determination of PRRS infection.** The serum analysis for viral concentration verified that all animals prior to inoculation were PRRS naive, and all animals became PRRS positive after inoculation.

**Virus titer and immune responses to PRRS inoculation.** Virus concentration increased sharply to  $10^{5.01}$  viruses/ml of serum at 4 days post-inoculation and then declined to a mean titer of  $10^{0.46}$  within 24 days. Twenty-five percent of the pigs had undetectable levels of viremia by 24 days.

Serum concentration of gamma-interferon responded in a similar pattern to viral titer; however, the AGP response was delayed by 6 to 8 days and then declined with time (Figure 1 and 2). The increase in serum gamma-interferon concentration demonstrates that macrophage activity was stimulated, and that the infection was significant enough to stimulate an immune response. The relationship of PRRS and AGP demonstrates the time necessary for an acute phase protein response to occur following a PRRS exposure.

### *Growth and feed intake response to PRRS inoculation.*

Pig gain and feed intake was depressed for 8 days post-inoculation and then began to increase (Figure 3 and 4).

**Regression analysis.** A multiple regression analysis was used to determine the relationship of serum concentration of virus, AGP, and  $\gamma$ -IFN, and pig weight on the associated four day pig gain and feed intake (Table 1). The data were analyzed using a backward stepwise regression procedure, which eliminated all effects that had a probability greater than .10. The components that accounted for a significant proportion of the variation in pig gain and feed intake are outlined in Table 1. The equation accounted for 48 and 54% of the variation in pig gain and feed intake, respectively.

$$\text{Pig gain} = -.5684 + (.1588 * \text{PW}) + (.0008 * \text{AGP}) - (.0105 * \text{PW} * \text{Titer}) - (.0207 * \text{Titer} * \text{Titer}) \quad R^2 = .69.$$

$$\text{Feed intake} = -.3721 + (.2290 * \text{PW}) + (.0850 * \text{Titer}) - (.0019 * \gamma\text{-IFN}) - (.0156 * \text{PW} * \text{Titer}) \quad R^2 = .80.$$

Pig gain and feed intake are reported as kilograms of gain and feed intake/pig for each four day period. Pig weight is reported as kilogram of body weight at the time of infection. Titers,  $\gamma$ -IFN, and AGP are reported as serum concentration of PRRS titer ( $10^x$  viruses/ml),  $\gamma$ -IFN (% protection to cells), and AGP ( $\mu$ g/ml). The linear and quadratic effects of pig weight, serum titer,  $\gamma$ -IFN, and AGP, and the two-way interactions were initially included in the regression analysis.

Each additional 10-fold increase in serum PRRS virus concentration was associated with a mean reduction of .559 kg in 4 day pig gain and .689 kg in 4 day pig feed intake. The specific magnitude of reduction in pig gain also was dependent in serum AGP status. As serum AGP concentration increased from 500 to 600  $\mu$ g/ml, pig gain was reduced by an additional .06 kg for each additional 10-fold increase of serum PRRS virus concentration. The magnitude of reduction in pig feed intake was dependent on  $\gamma$ -IFN, as well as, AGP concentration (Table 1).

**Table 1. Change in pig gain and feed intake for pigs infected with PRRS.**

Titer-Immune Factor Units	Pig Gain, kg/4days		Pig Feed, kg/4days	
	Coeff.	Prob.	Coeff.	Prob.
Intercept	2.94449	.01	6.35556	.01
Titers, $10^x$ /ml	-.55873	.01	-.68559	.01
$\gamma$ -IFN, 1% resistance	-.00257	.01	-.01261	.01
AGP, 1 $\mu$ g/ml	-.00241	.05	-.01140	.03
Titer X AGP	-.00067	.01	-.00069	.01
Titer X $\gamma$ -IFN			-.00300	.01
AGP X AGP			-.000007	.06

Based on these data, the magnitude of biological responses that occur in pigs infected with PRRS is directly related to the animal's serum virus concentration.

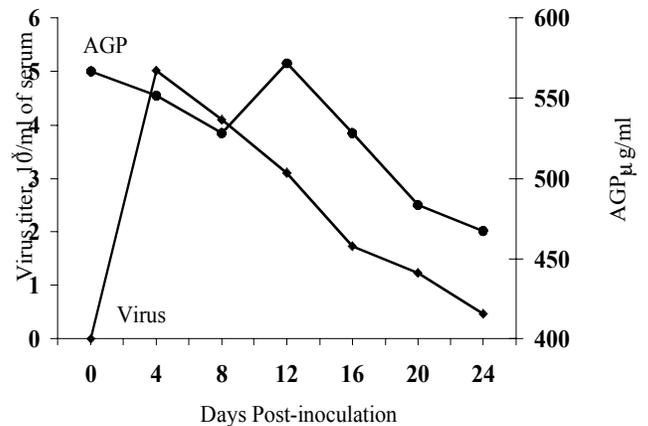
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**References**

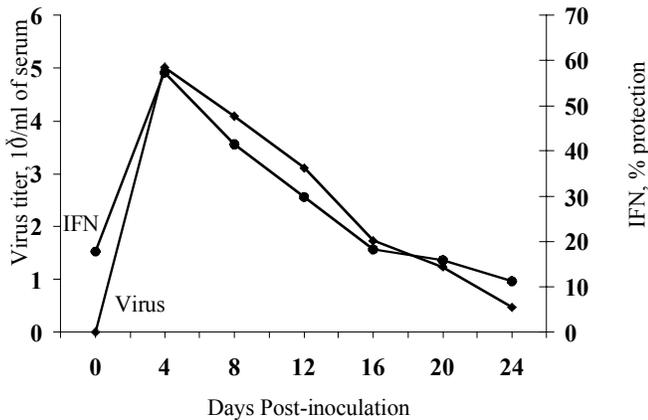
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**Virus titer and AGP**



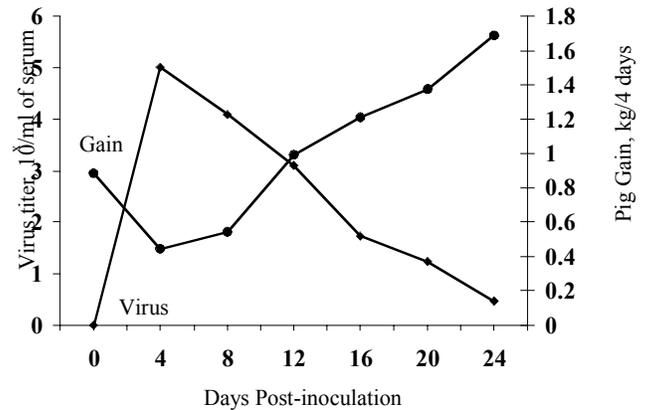
**Figure 2. Mean serum concentration of PRRS virus and AGP.**

**Virus titer and IFN**



**Figure 1. Mean serum concentration of PRRS virus and γIFN.**

**Virus titer and pig gain**



**Figure 3. Mean serum PRRS virus concentration and pig gain.**

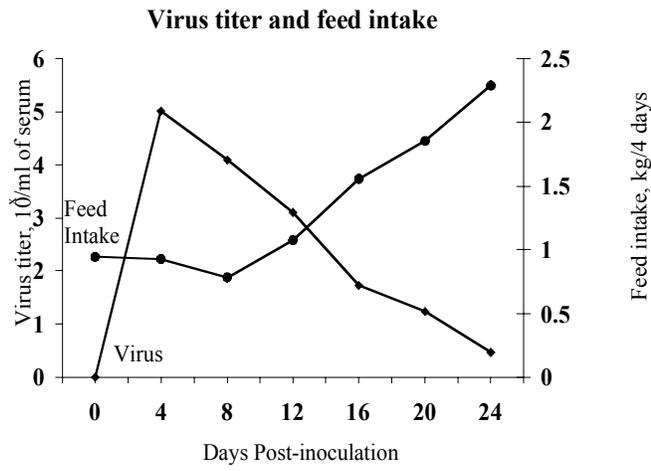


Figure 4. Mean serum PRRS virus concentration and pig feed intake.