Animal Science Publications   Animal Science

2009

Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection

A. B. Doeschl-Wilson
Scottish Agricultural College

I. Kyriazakis
University of Thessaly

Amy L. Vincent
Iowa State University, amyv@iastate.edu

Max F. Rothschild
Iowa State University, mfrothsc@iastate.edu

Eileen L. Thacker
Iowa State University, ethacker@iastate.edu

See next page for additional authors

Follow this and additional works at: http://lib.dr.iastate.edu/ans_pubs

Part of the Agriculture Commons, Animal Sciences Commons, Genetics Commons, Veterinary Microbiology and Immunobiology Commons, and the Veterinary Preventive Medicine, Epidemiology, and Public Health Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/ans_pubs/243. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection

Abstract

The response to infection from porcine reproductive and respiratory syndrome virus (PRRSV) for 2 genetically diverse commercial pig lines was investigated. Seventy-two pigs from each line, aged 6 wk, were challenged with PRRSV VR-2385, and 66 litter-mates served as control. The clinical response to infection was monitored throughout the study and pigs were necropsied at 10 or 21 d postinfection. Previous analyses showed significant line differences in susceptibility to PRRSV infection. This study also revealed significant line differences in growth during infection. Line B, characterized by faster growth rate than line A in the absence of infection, suffered more severe clinical disease and greater reduction in BW growth after infection. Correlations between growth and disease-related traits were generally negative, albeit weak. Correlations were also weak among most clinical and pathological traits. Clinical disease traits such as respiratory scores and rectal temperatures were poor indicators of virus levels, pathological damage, or growth during PRRSV infection. Relationships between traits varied over time, indicating that different disease-related mechanisms may operate at different time scales and, therefore, that the time of assessing host responses may influence the conclusions drawn about biological significance. Three possible mechanisms underlying growth under PRRSV infection were proposed based on evidence from this and previous studies. It was concluded that a comprehensive framework describing the interaction between the biological mechanisms and the genetic influence on these would be desirable for achieving progress in the genetic control of this economically important disease.

Authors

A. B. Doeschl-Wilson, I. Kyriazakis, Amy L. Vincent, Max F. Rothschild, Eileen L. Thacker, and L. Galina-Pantoja

Clinical and pathological responses of pigs from two genetically diverse commercial lines to porcine reproductive and respiratory syndrome virus infection[1]

A. B. Doeschl-Wilson,*[2] I. Kyriazakis,† A. Vincent,‡ M. F. Rothschild,§ E. Thacker,‡ and L. Galina-Pantoja#

*Scottish Agricultural College, Sustainable Livestock Systems, King's Buildings, West-Mains Road Edinburgh, EH9 3JG, United Kingdom; †Faculty of Veterinary Medicine, University of Thessaly, Trikalon 224, GR-43100, Karditsa, Greece; ‡Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames 50011; §Department of Animal Science, Center for Integrated Animal Genomics, Iowa State University, Ames 50011; and #Pig Improvement Company, 100 Bluegrass Commons Blvd., Hendersonville, TN 37075

ABSTRACT: The response to infection from porcine reproductive and respiratory syndrome virus (PRRSV) for 2 genetically diverse commercial pig lines was investigated. Seventy-two pigs from each line, aged 6 wk, were challenged with PRRSV VR-2385, and 66 littermates served as control. The clinical response to infection was monitored throughout the study and pigs were necropsied at 10 or 21 d postinfection. Previous analyses showed significant line differences in susceptibility to PRRSV infection. This study also revealed significant line differences in growth during infection. Line B, characterized by faster growth rate than line A in the absence of infection, suffered more severe clinical disease and greater reduction in BW growth after infection. Correlations between growth and disease-related traits were generally negative, albeit weak. Correlations were also weak among most clinical and pathological traits. Clinical disease traits such as respiratory scores and rectal temperatures were poor indicators of virus levels, pathological damage, or growth during PRRSV infection. Relationships between traits varied over time, indicating that different disease-related mechanisms may operate at different time scales and, therefore, that the time of assessing host responses may influence the conclusions drawn about biological significance. Three possible mechanisms underlying growth under PRRSV infection were proposed based on evidence from this and previous studies. It was concluded that a comprehensive framework describing the interaction between the biological mechanisms and the genetic influence on these would be desirable for achieving progress in the genetic control of this economically important disease.

Key words: genetic susceptibility, pig, porcine reproductive and respiratory syndrome, respiratory disease, response to infection

INTRODUCTION

Porcine reproductive and respiratory syndrome virus (**PRRSV**) causes a highly infectious disease of pigs that leads to interstitial pneumonia, reproductive failure, immunosuppression, and reductions in feed intake and performance. There is ample evidence for genetic variation in the response of animals to the infection (e.g., Halbur et al., 1998; Petry et al., 2005, 2007; Vincent et al., 2005, 2006) and for the existence of indicative biomarkers that could be targeted for selection (Galina-Pantoja et al., 2006; Petry et al., 2007). The immune response to PRRSV appears to be manifold, and there are many facets of resistance to the virus that could be targeted by genetic selection (Murtaugh et al., 2002). However, to date there is a lack of understanding of the relationships between traits associated with PRRSV susceptibility and performance traits, such as growth in the face of PRRSV infection.

Porcine reproductive and respiratory syndrome virus infection is commonly known to have a negative impact

on growth, but the degree of the impact appears to vary between and within pig lines or breeds (Greiner et al., 2000; Petry et al., 2005, 2007) The underlying mechanisms for this relationship are still poorly understood. It can be envisaged that genetic resistance to PRRSV may influence performance in positive (e.g., less impact of the virus on the biological systems) and negative (e.g., the resistant animal diverts more resources and effort to the immune mechanisms rather than growth) ways.

The objectives of this study were to quantify the effect of PRRSV infection on performance and disease traits for 2 genetically diverse commercial lines of pigs and to assess the manner in which the lines differ in their response to infection. We further investigated the relationship between performance and diverse disease traits, and how it changed over the time course of infection. The study also aimed to contribute toward a better biological understanding of the mechanisms that determine how animals cope with the infection.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because the data were obtained from an existing database (Vincent et al., 2006).

The data set used in the present study constitutes a subset of the data set used by Vincent et al. (2006). Records from only 3 out of the 4 independent replicates previously reported were used because the first trial did not include a complete set of the traits considered in the analysis of the present study.

Experimental Design

Two hundred ten pigs, aged between 2 and 4 wk, from 2 genetically diverse commercial lines of pigs were procured from a PRRSV-free farm in 3 independent replicates. Line A was derived from a Large White population, whereas line B was derived from a Pietrain composite.

Line A pigs consisted of 103 offspring from 5 sires and 19 dams, and line B consisted of 107 offspring from 7 sires and 21 dams. Each trial replicate contained between 33 and 36 animals from each line. At approximately 6 wk of age, designated here as 0 d postinfection (**dpi**), 24 animals of each line in each trial were infected intranasally by a 5-mL dose of a high-virulence, low-passage strain (VR-2385) of PRRSV inoculum at 10^5 to 10^6 tissue culture infective dose 50 (**TCID$_{50}$**/mL) as described in Halbur et al. (1995, 1996). Challenged pigs of the same litter were sorted randomly into different pens of 8 pigs each. The remaining pigs (n = 66) were used as experimental negative controls; these were housed in a separate isolation facility with identical conditions, during each respective replicate. One-half of the challenge and control pigs from each line were necropsied at 10 dpi, the remaining one-half at 21 dpi.

Traits Measured

Traits and measurement techniques were described previously (Vincent et al., 2006). The techniques are briefly described below.

Performance and Clinical Signs. All pigs were weighed 5 d before infection and before slaughter at 10 or 21 dpi. From the time point of infection onwards animals were observed daily up to 10 dpi and subsequently every other day for clinical symptoms. Rectal temperatures were measured, and the degree of respiratory distress was assessed on the same days. The latter was assessed using a previously described method for evaluating the degree of dyspnea, tachypnea, or both (Halbur et al., 1996) on a scale from 1 to 6, where 0 denotes normal; 1 denotes mild dyspnea, tachypnea, or both when stressed and 2 when at rest; 3 denotes moderate dyspnea, tachypnea, or both when stressed and 4 when at rest; and 5 denotes dyspnea, tachypnea, or both when stressed and 6 when at rest.

Blood (18 mL, vacuum container tube) was collected from all pigs before and immediately after challenge to monitor the presence of PRRSV antibodies using a commercially available ELISA test (HerdChek: PRRS, IDEXX Laboratories, Westbrook, ME). Blood samples were also collected from each pig on necropsy day for assessing virus titers.

Lesions. After necropsy, lung and other tissue samples were taken for macroscopic and microscopic examination. Lungs were removed from each animal, and individual lobes were evaluated for macroscopic lesions, yielding an estimate for the percentage of the total lung affected by grossly visible lesions. Bronchoalveolar lavage (**BAL**) fluid was also collected from the lungs for virus quantification. Lung sections were examined for the degree of interstitial pneumonia (**IPS**) using a score from 0 to 6.

Virus Titration and Quantification. Standard aliquots of each BAL and serum sample were titrated in triplicate and screened for PRRSV using an indirect immunofluorescence assay. The TCID$_{50}$ was calculated using the Reed-Muench accumulative method (Coligan et al., 1996).

Statistical Analysis

A pig was classified as febrile if its rectal temperature was above the 95th percentile of the temperature of the control animals from the same line at that day. For every animal, the number of febrile days and the number of days with elevated respiratory score (**R-score** > 0) between 0 dpi and necropsy day were calculated. Actual rectal temperature and respiratory scores were also totaled within the time period m (**Tcum**, **Rcum**). To filter out the high day-to-day variation of temperature and respiratory scores, a moving average was calculated from 3 consecutive temperature and respiratory score measures. Virus titers were log (base 10) transformed.
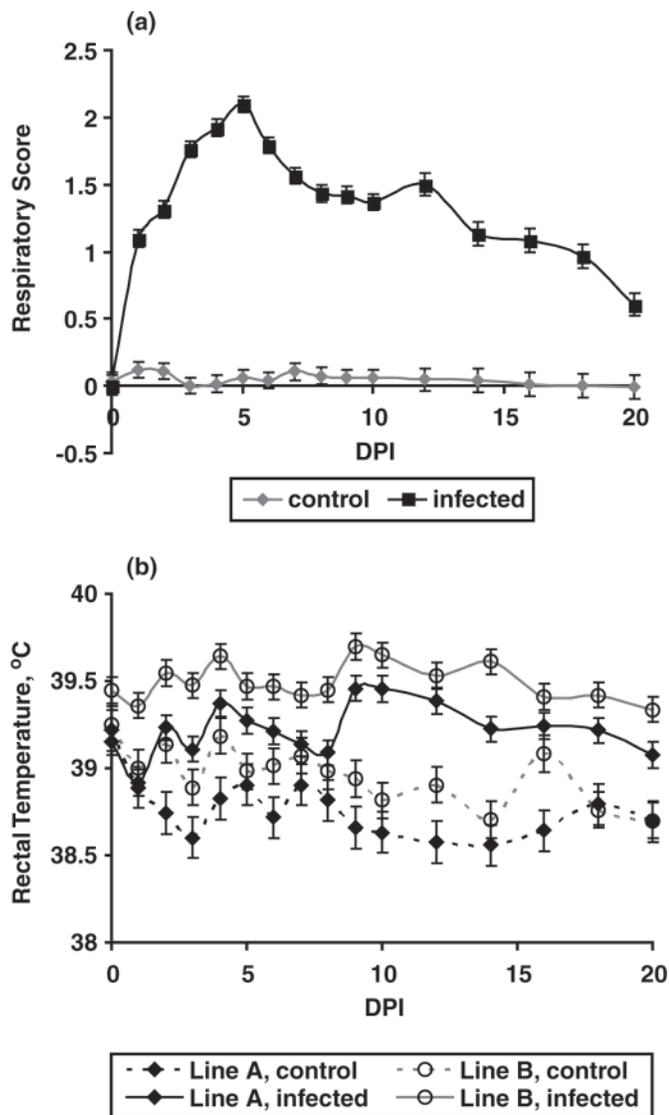
Figure 1. (a). Daily least squares means of respiratory scores for infected and control animals. Line differences were not statistically significant ($P < 0.4$). (b) Daily least squares mean rectal temperatures × treatment group and line. Both treatment and line had statistically significant effects over the trial period ($P < 0.001$), but line × treatment interaction was not statistically significant ($P < 0.2$). DPI = days postinfection.

Endpoint data and respiratory and rectal temperature profiles were analyzed using REML mixed model analysis (PROC MIXED, SAS Inst. Inc., Cary, NC). Treatment (control, infected), line, pen, sex, and trial replicate were assumed to be fixed effects, whereas sire and litter were assumed random effects. The fixed effect of treatment was omitted from the models for viremia and lesions because measurements were only taken on infected animals. Traits measured at different necropsy dates were analyzed as separate traits.

For the daily respiratory and temperature measures, dpi was entered into the models as a repeated measure. Relationships between diverse disease and performance traits for infected animals within and between periods were assessed based on Spearman rank-order correla-

tions (PROC CORR, SAS Inst. Inc.). The threshold $P < 0.05$ was used for statistical significance.

RESULTS

Clinical Signs, Course of Infection, and Line Differences

All animals were serologically negative before challenge, and uninfected control animals remained so throughout the length of the trials. The majority (87%) of control animals had 0 respiratory scores throughout the trial, with the remaining animals having score 1 for some days. Infection had a statistically significant impact on all performance traits, clinical signs, and lesions ($P < 0.0001$).

Respiratory Scores

Signs of respiratory distress were visible from 24 h after challenge (Figure 1a). Respiratory scores of infected animals generally increased to a maximum score (maximum R-score = 2 or 3 for most animals) within the first 5 d of infection and gradually decreased toward the end of the trial (Figure 1a). Some, but not all, of the challenged animals had R-scores comparable with those of control pigs by the end of the trial (20 dpi). The least squares mean (**LSM**) for R-score of infected animals at the end of the trial was significantly greater than the score at the beginning ($P < 0.0001$). All of the infected animals that were necropsied at 21 dpi showed respiratory distress (i.e., R-score > 0) for a duration between 11 and 15 d out of the 20 trial days (76% had R-score > 0 for 14 or 15 d). There were no effects of line on R-score or any interactions between line and infection. The same applied to the number of days with respiratory distress.

Fever

Daily LSM rectal temperatures were significantly greater ($P < 0.0001$) for the infected than control animals from 24 h after challenge and remained so over the entire trial period (Figure 1b). All infected animals were classified as febrile for a time period of between 2 and 14 d out of the 20 trial days. The LSM for number of febrile days of infected animals necropsied at 21 dpi was 6.55 d (SEM = 0.57) across all trials. Rectal temperatures were consistently greater ($P < 0.001$) for line B than line A animals, but this was equally true for control and infected animals (Figure 1b). The line × treatment interaction was not statistically significant for rectal temperatures, cumulative temperatures, or number of days with febrile temperature.

BW Gain

Infection had a significant impact on BW gain ($P < 0.0001$), and the impact became more apparent when

inspected over the longer time period from 5 d before infection until 21 dpi (Figure 2). The LSM difference in BW gain between infected and control animals corresponding to the time period of 5 d before infection until 10 dpi was 2.53 ($\pm$0.75) kg ($P < 0.002$). The difference increased to 5.72 ($\pm$0.78) kg ($P < 0.0001$) when considered over the longer time period up to 21 dpi.

As illustrated in Figures 2a and b, the pig lines differed not only in their BW gain, but also in the reduction of growth due to the infection (i.e., significant line $\times$ treatment interaction for 21 dpi). At 21 dpi, noninfected animals of line B had grown on average 9.4 ± 1.8 kg more in BW than noninfected line A animals. For the infected animals, however, the difference between the lines was only 4.2 ± 1.6 kg. Infection caused a reduction in BW gain of 8.3 ± 1.0 kg for line B animals over the time period between 5 d before 21 dpi, but of only 3.1 ± 1.3 kg for line A animals. The effects for the shorter time period between 5 d before 10 dpi were similar, but weaker (no statistically significant line $\times$ treatment interaction, Figure 2a).

Virus Titers

At 10 dpi, virus titers in BAL and serum of the challenged animals were between $10^{3.5}$ and $10^{6.3}$ TCID$_{50}$ and between $10^{2.8}$ and $10^{5.3}$ TCID$_{50}$, respectively. At 21 dpi, the virus titer had dropped significantly (Figures 3a and b) in all animals and for 37% of animals to undetectable levels. With all trials and lines pooled, the LSM virus titer in BAL dropped from $10^{5.10 \pm 0.08}$ at 10 dpi to $10^{1.86 \pm 0.19}$ TCID$_{50}$ at 21 dpi. The equivalent LSM virus titers in serum were $10^{4.08 \pm 0.06}$ and $10^{1.46 \pm 0.17}$ TCID$_{50}$, respectively. Viral load declined more rapidly to undetectable levels in serum than in BAL, at 21 dpi 28% of animals still had detectable virus titer in BAL, but not in the blood.

Similarly, line differences in the virus titer in BAL and serum at dpi 10 were not found to be statistically significant and were inconsistent between the trials (Figure 3b). The same was true for the BAL virus titer at 21 dpi. However, at 21 dpi line A animals had significantly greater viremia than line B animals consistently in all trials (Figure 3b). At that time, blood virus titer had fallen below a detectable level for 75% of line B animals compared with 56% of line A animals, indicating that line B animals managed to clear the virus from the blood more rapidly than animals of line A.

Pneumonia

Infection with PRRSV caused significant macroscopic lung lesions in all challenged animals. The LSM of percentage of lung affected by macroscopic lesions at 10 dpi were 38.29 ($\pm$3.50)% and 48.17 ($\pm$3.41)% for lines A and B, respectively (Figure 3c), and the difference was found to be statistically significant ($P < 0.05$). At 21 dpi, the lesions had reduced significantly (LSM = 12.0 ± 1.09% for both lines pooled), but in contrast
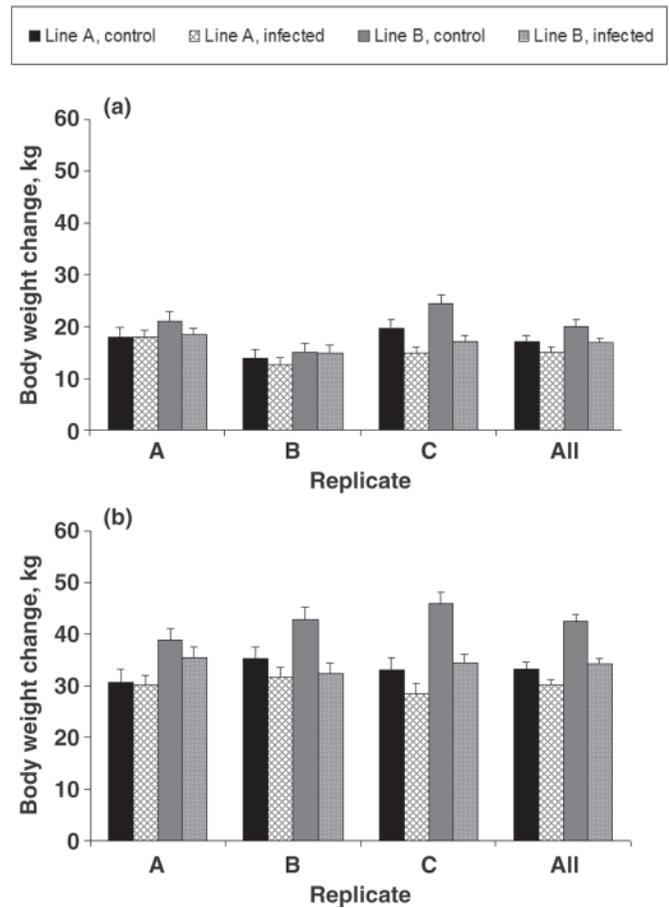

Figure 2. Least squares mean BW changes for pig lines A and B from 5 d before infection until 10 d postinfection (a) or 20 d postinfection (b). Line and treatment effects were statistically significant ($P < 0.05$) at both 10 and 21 d postinfection. Line $\times$ treatment interactions were statistically significant ($P < 0.05$) only at 21 d postinfection.

to the virus titers, were still detectable in all except 1 animal. The difference between lines had declined to a nonsignificant level (Figure 3c).

Microscopic IPS were consistent with the macroscopic lung lesions (Figure 3d). At 10 dpi, all infected animals had microscopic IPS between 2 and 6 [LSM = 3.75 (0.18) for line A and LSM = 4.25 (0.18) for line B, pdiff < 0.06]. At 21 dpi, the IPS range had declined to scores between 1 and 4 [LSM = 1.73 (0.14) for line A and LSM = 2.19 (0.14) for line B], but the difference between the lines remained statistically significant (pdiff < 0.04). Control animals had IPS between 0 and 2 for either necropsy date, with the majority of animals having a score of 1.

Relationship Between Disease Traits

The Spearman rank-order correlation coefficients between the diverse clinical traits are presented in Table 1, along with lesions and performance traits measured on infected animals from both pig lines pooled.

Relationship Between Fever and Respiratory Scores. Correlations between traits related to fever and respiratory distress were consistently posi-
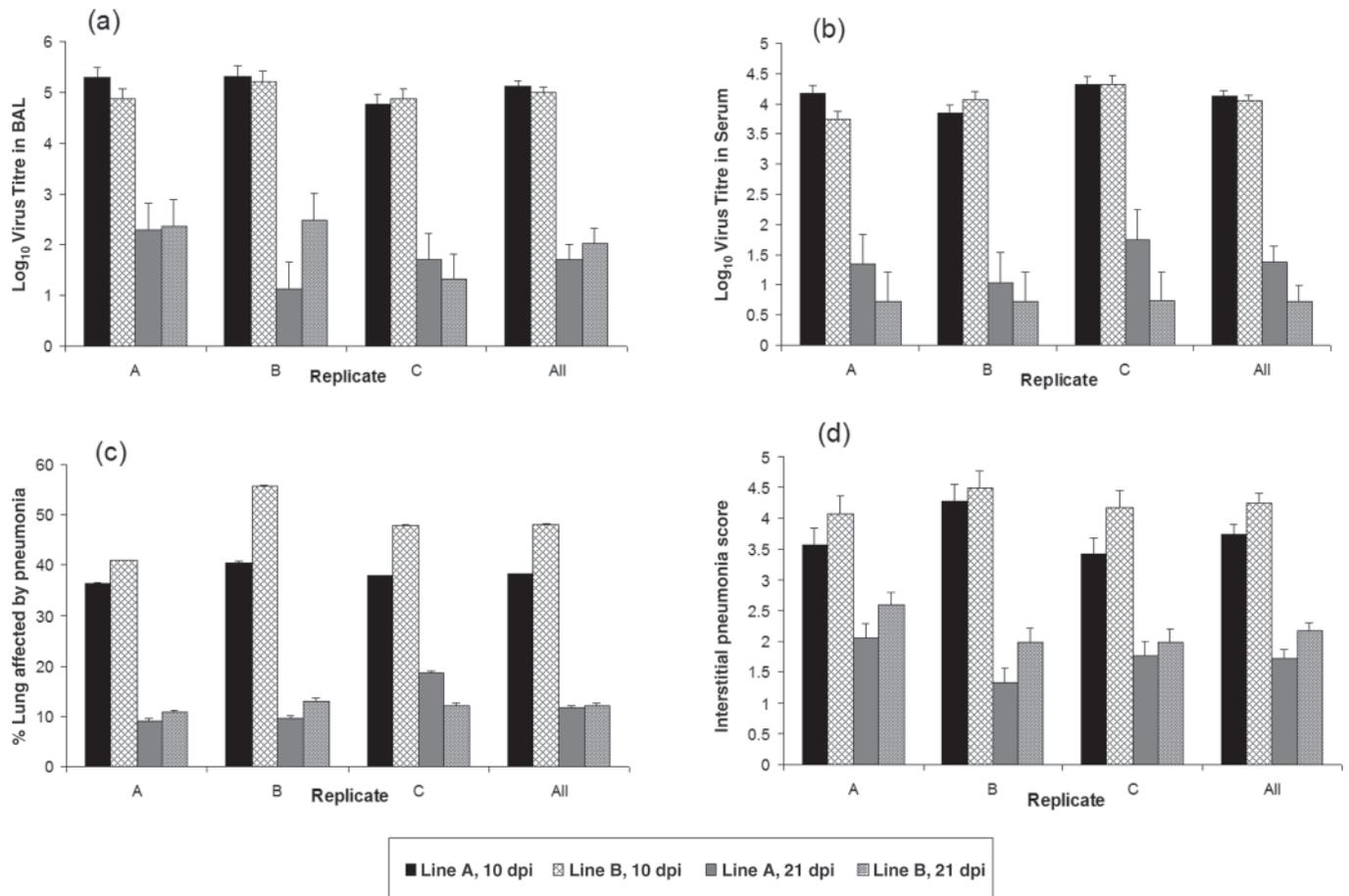
Figure 3. Least squares means for (a) virus titer (log 10) in bronchoalveolar lavage (BAL), (b) virus titer (log 10) in serum, (c) percentage of lung affected by pneumonia, and (d) interstitial pneumonia scores for pig lines A and B at 10 and 21 d postinfection.

tive, although not always significantly different from 0. The severity of fever and respiratory distress were moderately related [r = 0.29 (P < 0.04) at 10 dpi and r = 0.36 (P < 0.03) at 20 dpi for Tcum and Rcum]. The number of febrile days was only significantly correlated with the number of days with elevated respiratory scores after 10 dpi (r = 0.46), but not after the full trial period of 20 d. The duration of the febrile period between start of infection and necropsy date and cumulative temperatures were positively correlated with the smoothed endpoint temperature near the necropsy date. The correlations were strong at 10 dpi (r = 0.75, P < 0.001, for number of febrile days and r = 0.80, P < 0.001, for cumulative temperatures), indicating that repeated temperature measurements may not be required at the early stages of infection. At 20 dpi, correlations were still significant, but weaker (r = 0.49, P < 0.01, for number of febrile days and r = 0.65, P < 0.005, for cumulative temperatures), reflecting the recovery of many animals by 20 dpi. In contrast, the severity of respiratory distress at the endpoint date was only moderately related to cumulative respiratory scores (r = 0.63, P < 0.01, and r = 0.52, P < 0.01, for 10 and 20 dpi, respectively) and only significantly related to the length of period with respiratory distress when mea-

sured over a sufficiently long time period (r = 0.62, P < 0.01, for animals necropsied at 21 dpi).

As indicated by the diagonal values in Table 1, a generally strong positive correlation across time was found for the individual temperature and respiratory summary traits (i.e., number of days, cumulative scores).

Relationship Between PRRSV Titers in BAL and Serum and Lesions. Virus titers in BAL and in serum were only significantly related at 21 dpi (r = 0.47), but not at 10 dpi, where a high virus load was detected in all infected animals. A moderately strong positive relationship was found between the percentage of lung affected by macroscopic lesions and the microscopic IPS, with a stronger correlation at 10 dpi (r = 0.52, P < 0.01) than at 21 dpi (r = 0.33, P < 0.01). Both macroscopic and microscopic pneumonia indicators were positively related to virus titer in BAL at both sampling points, with correlations ranging from r = 0.33 to r = 0.46, P < 0.05, Table 1). Serum virus titers were, however, only significantly related to macroscopic lung lesions at 21 dpi (r = 0.27, P < 0.01).

Relationship Between ADG and Clinical Signs and Lesions. The ADG was negatively correlated with all clinical signs and lesions examined, although the majority of correlations were not statis-

Table 1. Spearman rank correlations between traits recorded in pigs infected with porcine reproductive and respiratory syndrome virus (PRRSV)[1]

Trait[2]	Performance	Clinical sign						PRRSV titer		Lesions	
	ADG	Fdays	Rdays	Tcum	Rcum	sT_nec	sR_nec	BAL	SER	Macro	Micro (IPS)
Performance											
ADG	NA*	−0.01	−0.38	0.04	−0.13	−0.07	0.11	−0.04	0.06	−0.24	−0.13
Clinical sign											
Fdays	−0.05	0.89*	0.46	0.88	0.17	0.75	0.04	−0.10	0.02	0.22	−0.09
Rdays	−0.11	0.14	0.74*	0.47	0.38	0.49	0.10	−0.14	0.02	0.12	0.17
Tcum	−0.02	0.78	0.34	0.96*	0.29	0.80	0.15	−0.12	0.03	0.24	−0.10
Rcum	−0.12	0.19	0.64	0.36	0.90*	0.28	0.63	0.16	−0.17	0.25	0.14
sT_nec	−0.01	0.49	0.18	0.65	0.17	0.47*	0.17	−0.02	−0.03	0.24	0.02
sR_nec	−0.02	−0.07	0.62	0.18	0.52	0.12	0.21*	0.03	−0.13	0.11	0.13
PRRSV titer											
BAL	−0.26	−0.22	−0.19	−0.10	−0.22	0.22	−0.06	NA*	−0.08	0.37	0.33
SER	−0.40	−0.13	−0.03	−0.11	−0.10	−0.04	−0.03	0.47	NA*	−0.09	−0.09
Lesions											
Macro	−0.09	0.00	0.07	0.15	−0.08	0.30	0.01	0.46	0.27	NA*	0.52
Micro (IPS)	−0.03	0.05	−0.24	0.11	−0.11	0.22	0.03	0.46	0.17	0.33	NA*

[1]Values above the diagonal refer to records collected immediately before or after necropsy at 10 d postinfection (dpi); values below the diagonal refer to records collected immediately before or after necropsy at 21 dpi. Values on the diagonal (marked with *) represent correlations between the same trait measured at 10 and 21 dpi, respectively, based on records collected on infected animals necropsied after 21 dpi. These correlations could only be calculated for the traits for which repeated measurements were taken (i.e., respiratory scores and rectal temperature). NA = not applicable. All correlations with magnitude above 0.22 were significantly different from zero ($P < 0.05$).

[2]Fdays and Rdays = number of days with elevated temperature and respiratory scores, respectively; Tcum and Rcum = cumulative temperature and respiratory scores; sT_nec and sR_nec = smoothed average temperature/respiratory score records 3 d before necropsy; BAL and SER = virus titer in bronchoalveolar lavage and serum; Macro = percentage of lung affected by macroscopic lesions; and Micro (IPS) = interstitial pneumonia scores.

tically significantly different from zero (Table 1). For the short time period up to 10 dpi, growth was only significantly correlated with the number of days with elevated respiratory scores (r = −0.38, $P < 0.02$) and not with other clinical signs or lesions. For the longer time period lasting until 21 dpi, growth was not significantly related to severity or duration of fever or respiratory distress.

The relationship between growth and virus titers in BAL or serum was only significant at 21 dpi (r = −0.26, $P < 0.02$, and r = −0.40, $P < 0.01$, respectively) and was not significant at 10 dpi. The lesions indicators of pneumonia were not significantly related to growth at either time period.

Relationship Between Fever, Respiratory Scores, PRRSV Titers, and Lesions. At 10 dpi, rectal temperatures were not significantly related to BAL or serum virus titers (Table 1). At 21 dpi, a significantly strong relationship between the number of febrile days and the rectal temperature near necropsy with the BAL virus titer was found, however (r = −0.22 and r = 0.22, $P < 0.02$, respectively), suggesting not only that rectal temperature is a moderate indicator of virus titer in BAL, but also that animals that reacted to the infection by increased body temperature over a longer period of time were likely to have decreased virus levels at the end of the period. The relationship with the temperature traits, however, was not significant for the serum virus titer. Indicators of respiratory distress were found significantly related to virus titer in BAL or serum at either sampling time. Macroscop-

ic lung lesions were positively related to indicators of elevated rectal temperatures and respiratory distress, but the correlations were generally weak (i.e., r ≤ 0.30; Table 1).

Line Differences. Correlations were also calculated separately for each individual line (Table 2). Although the signs of the correlations were generally consistent between lines, the strength of the correlations often varied. At 10 dpi, correlations between ADG and lesions traits were significantly stronger for line B than line A (e.g., correlation between ADG and macroscopic lesions was −0.04 for line A and −0.55 for line B, pdiff < 0.04). At 21 dpi, the situation reversed, and correlations between ADG and diverse clinical signs or lesions were significantly stronger for line A than line B. Similarly, at 10 dpi, correlations between BAL virus titer and lesions were significantly stronger for line B (r = 0.61 and r = 0.51 for macroscopic and microscopic lesions, respectively) than line A (r = 0.16 and r = 0.03 for macroscopic and microscopic lesions, respectively). Correlations between serum virus titer and lesions score were not statistically significant for either line. At 21 dpi, correlations between BAL virus titer and macroscopic lesions were still strong for line B in comparison with those of line A (r = 0.67 for line B and r = 0.16 for line A, pdiff < 0.01), but correlations between BAL virus titer and microscopic lesions were significantly stronger for line A than for line B (r = 0.67 and r = 0.16, respectively, pdiff <0.01). Also, a moderately strong relationship between serum virus titers and lesions was found for line A at 21 dpi (r = 0.48

Table 2. Spearman rank correlations between traits recorded in porcine reproductive and respiratory syndrome virus (PRRSV)-infected pigs of lines A and B, respectively[1]

Trait[2]	Pig line	Performance ADG	Clinical sign Fdays	Rdays	Tcum	Rcum	sT_nec	sR_nec	PRRSV titer BAL	SER	Lesions Macro	Micro (IPS)
Performance												
ADG	A	NA*	−0.09	−0.40	−0.04	−0.05	−0.08	−0.02	0.06	0.37	−0.04	−0.03
	B	NA*	0.08	−0.38	−0.03	−0.41	−0.10	−0.10	0.03	−0.20	−0.55	−0.30
Clinical sign												
Fdays	A	−0.15	0.89*	0.55	0.95	0.25	0.77	0.02	−0.20	−0.06	0.25	−0.12
	B	−0.05	0.88*	0.33	0.90	0.10	0.76	−0.10	−0.10	−0.20	0.08	0.13
Rdays	A	−0.12	0.36	0.96*	0.57	0.38	0.55	0.02	−0.29	−0.27	0.07	0.17
	B	−0.18	0.10	0.95*	0.39	0.37	0.42	0.20	0.04	0.34	0.1	0.13
Tcum	A	−0.28	0.91	0.48	0.92*	0.29	0.76	0.06	−0.20	0.07	0.18	−0.18
	B	−0.06	0.86	0.17	0.96*	0.26	0.87	0.03	−0.15	0.05	0.16	−0.12
Rcum	A	−0.21	0.23	0.68	0.30	0.92*	0.32	0.58	−0.03	−0.50	0.08	−0.02
	B	−0.08	0.15	0.54	0.37	0.90*	0.18	0.64	0.40	0.14	0.43	0.33
sT_nec	A	−0.33	0.62	0.28	0.64	0.27	0.54	0.17	−0.02	−0.24	0.14	−0.05
	B	−0.07	0.47	0.10	0.45	0.13	0.09	0.18	−0.01	0.20	0.25	0.10
sR_nec	A	−0.20	−0.07	0.66	0.04	0.70	0.17	0.37	−0.29	−0.27	−0.10	−0.08
	B	−0.04	−0.16	0.54	−0.10	0.28	−0.21	0.07	0.32	0.19	0.37	0.35
PRRSV titer												
BAL	A	−0.51	−0.10	−0.33	−0.12	−0.24	0.09	−0.01	NA	−0.14	0.16	0.03
	B	−0.11	−0.32	−0.20	−0.30	−0.25	0.28	−0.20	NA	0.12	0.61	0.55
SER	A	−0.42	0.10	−0.09	0.12	−0.13	0.15	−0.04	0.72	NA	−0.09	−0.28
	B	−0.30	−0.38	0.05	−0.20	0.03	−0.09	0.09	0.25	NA	−0.08	0.10
Lesions												
Macro	A	−0.29	0.18	−0.04	0.28	−0.15	0.25	−0.18	0.29	0.48	NA	0.24
	B	−0.10	−0.18	0.14	−0.15	−0.08	0.28	0.10	0.61	0.16	NA	0.76
Micro (IPS)	A	−0.44	0.03	−0.25	−0.05	−0.15	0.02	−0.10	0.67	0.57	0.38	NA
	B	−0.23	0.08	−0.39	−0.09	−0.24	0.19	0.01	0.16	−0.22	0.18	NA

[1]Values above the diagonal refer to records collected immediately before or after necropsy at 10 d postinfection (dpi), values below the diagonal refer to records collected immediately before or after necropsy at 21 dpi. Values on the diagonal (marked with *) represent correlations between the same trait measured at 10 dpi and 21 dpi, respectively, based on records collected on infected animals necropsied after 21 dpi. These correlations could only be calculated for the traits for which repeated measurements were taken (i.e., respiratory scores and rectal temperature). NA = not applicable. All correlations with magnitude above 0.29 were significantly different from zero ($P < 0.05$).

[2]Fdays and Rdays = number of days with elevated temperature and respiratory scores, respectively; Tcum and Rcum = cumulative temperature and respiratory scores; sT_nec and sR_nec = smoothed average temperature/respiratory score records 3 d before necropsy; BAL and SER = virus titer in bronchoalveolar lavage and serum; Macro = percentage of lung affected by macroscopic lesions; and Micro (IPS) = interstitial pneumonia scores.

and r = 0.57 for macroscopic and microscopic lesions, respectively), whereas the relationship was weak at the same time point for line B (r = 0.16 and r = −0.22 for macroscopic and microscopic lesions, respectively).

DISCUSSION

In this study, the relationships between various disease-related traits and between growth and disease-related traits were examined for 2 genetically diverse commercial pig lines when challenged with PRRSV. Historically, line A had been selected mainly for reproductive performance, robustness, and growth, whereas line B had been selected mainly for lean growth rate and feed efficiency. Previous studies demonstrated significant line differences in their susceptibility to PRRSV (Vincent et al., 2005, 2006). The present study revealed that significant line differences also exist for growth response following PRRSV challenge. Line B, characterized by a faster growth rate than line A in the absence of infection, suffered significantly greater reduction in BW growth after infection. Line B also

showed more severe clinical signs and lesions than line A. Correlations between growth and disease traits were generally negative, albeit weak. The strongest (negative) correlations with growth rate were found for the virus titer in BAL or serum at 21 dpi, where virus levels had already decreased to undetectable levels in many animals. The results thus suggest that reduction in growth when measured during the short time span of an acute infection (i.e., 10 d) is a poor indicator of the severity of infection and that reductions in growth may be more strongly related to the duration than the severity of infection.

In general, weak correlations were also found among individual clinical signs and lesions. Respiratory scores and rectal temperatures measured at any stage during the infection appear to be poor indicators of virus levels in BAL and serum or of the impact of PRRSV infection on growth and lesions. The relationship between rectal temperatures at the early stage of the infection and the lesions, as well as growth measured at 10 and 21 dpi, was generally weak, thus providing no indication that a febrile response at the early stage of PRRSV infection

has any benefits for the severity or the duration of the infection (Hart, 1988; Blatteis, 2003). Only temperatures close to 21 dpi were significantly correlated ($P < 0.05$) with macroscopic lung lesions (at both necropsy dates), suggesting that elevation in body temperature and lung tissue damage may share some common underlying biological mechanisms (Hart, 1988).

The 2 pig lines appeared to differ in the strength, but not in the direction of the relationship between performance and disease traits. At 10 dpi, correlations between growth rate and the lesions were significantly stronger for line B, but the situation reversed after 21 dpi, at which time correlations were significantly stronger for line A. Also, the relationships between virus titers and lesions were considerably stronger for line A than for line B at that time.

Line or breed differences in the response of pigs to PRRSV infection have been examined previously. Halbur et al. (1998) found that PRRSV-infected Duroc pigs had less ADG and more severe PRRSV-induced lung lesions than Meishan pigs. Petry et al. (2005) compared the impact of PRRSV infection on growth and disease traits for a Large White-Landrace composite line (**NEI**) that had been selected for litter size and a Hampshire-Duroc cross (**HD**) selected for rate and efficiency of lean growth. Uninfected HD pigs had greater ADG than uninfected NEI pigs, but PRRSV infection resulted in HD pigs having less ADG than infected NEI pigs. Similar to the response seen in our study, the pigs from the lean HD line had greater incidence of interstitial pneumonia and virus concentration in blood, lung, and bronchial lymph nodes than NEI pigs. All 3 studies thus consistently demonstrate that pigs from lines or breeds selected for lean growth rate appear to be less resistant to PRRSV infection and experience greater reduction in BW growth than pigs from lines selected for reproductive traits.

The typical commercial pigs are a genetic composite of lines selected for lean growth and reproductive performance. With the recent advances in genomics and the likely use of a highly dense SNP chip in the near future, it will soon be possible to better understand which specific genes are associated with and hence affect biological functions that are affected by PRRSV. Marker-assisted selection may enable selection for more robust pigs that perform better in the face of PRRSV challenge but that still retain desirable characteristics such as lean growth and reproductive performance.

Growth and severity of infection have also been found to be consistently negatively related across different PRRSV challenge studies. Greiner et al. (2000) and Petry et al. (2005) both reported a negative relationship between serum PRRS virus concentration and BW growth. However, they also found that the strength of the relationship varied throughout the stage of infection, with the strongest correlation/greatest reduction in growth occurring at the later infection stages when systemic virus concentration was less. It remains to be shown whether the change in strength of the relationship is an artifact of a different variation in growth rate at different time periods or is due to a direct relationship between growth rate and some immune process that is mainly active at late infectious stages. Johnson et al. (2004) have reported that the impact of infection on growth was strain dependent. In their challenge study, infection with the virulent PRRSV strain MN184 produced the most severe infection of the longest duration and resulted in significantly less BW gain over a 49-d period than that of pigs infected with less virulent strains. Petry et al. (2005) also reported generally weak correlations between growth rate and diverse clinical disease traits (e.g., rectal temperature) and lesions (e.g., macroscopic and microscopic lesions, virus titer in lung, and bronchial lymph nodes) and also between diverse clinical signs and lesions. The weak associations indicate that the different disease traits relate to different components of the immune response. Estimates of genetic correlations, which require more data than were available for this project, would provide valuable insight on this matter.

Although the present data set only contains repeated measurements for the clinical disease indicators (i.e., rectal temperature and respiratory scores) and for the other traits at 2 different time points, the statistical results nevertheless indicate that relationships between traits are not stable over time. These relationships likely change as a result of different disease-related mechanisms operating on different time scales. For example, virus concentration in serum and BAL were significantly related at 21 dpi, but not at 10 dpi, where virus levels were substantially greater, suggesting different time trends for the virus dynamics at different locations. Indeed, viral loads in serum are reported to peak as early as 4 dpi (Greiner et al., 2000), whereas virus load in the lung peaks at 7 to 9 dpi (Labarque et al., 2003). However, Petry et al. (2005) found moderately strong positive correlations between virus concentrations in blood, lung, and lymph nodes at 14 dpi. These reports, combined with the results of the present study, indicate that animals with greater PRRSV concentration in blood at later infectious states are also likely to have greater virus concentrations in other tissues for which in vivo samples are difficult to obtain. Time trends, and hence the relationships between traits, may also depend on the PRRSV strain. For example, Johnson et al. (2004) showed that some of the more virulent strains lead to greater viremia levels at the early stages of infection, but also a faster decline due to increased antibody titers.

It is well documented that PRRSV has a negative effect on growth, but as illustrated above, the magnitude of this effect varies between and within lines and breeds. The analyses suggest that selection for production or decreased PRRSV susceptibility may have a substantial impact on this relationship and that therefore some understanding of the underlying mechanisms governing this relationship would be desirable. The growth-susceptibility relationship may be determined

by at least 3 possible underlying mechanisms that are under partial genetic control: i) PRRSV infection leads to reduction in feed intake with direct consequences on growth, ii) immune response has a direct effect on growth through common genetic pathways other than those controlling feed intake, and iii) growth and immune response compete for the same resources leading to a trade-off between growth and immunity.

Evidence exists in the literature in support of all 3 of these mechanisms. For example, immunological studies revealed that PRRSV infection (combined with *Mycoplasma hypneumonieae*) induces increased production in proinflammatory cytokines including IL-1β and IL-6 (Escobar et al., 2004; Thanawongnuwech et al., 2004) These cytokines not only coordinate and activate the adaptive immune response (Van Reeth et al., 1999; Van Reeth and Nauwynck, 2000) but are also known to trigger reduction in feed intake and to downregulate the GH IGF-I axis (Roberts and Almond, 2003; Escobar et al., 2004). By correcting BW gain for feed intake in their covariance analysis, Escobar et al. (2004) concluded that the reduction in BW gain of PRRSV-infected pigs was mainly due to reduction in feed intake, thus pointing toward a big role for the first of the above listed mechanisms in response to PRRSV infection. However, Escobar et al. (2004) also reported an increase in the expression of myostatin in the muscle after PRRSV infection, which may have been activated by proinflammatory cytokines. Myostatin is known to be a negative regulator of muscle mass and thus growth in various species (McPherron et al., 1997; Sharma et al., 1999). Also, in a separate experiment, Roberts and Almond (2003) found no significant differences in feed intake between PRRSV infected and uninfected control animals after 11 dpi, but IGF-I depression of infected pigs continued through to 28 dpi. The results therefore suggest that PRRSV infection, independent of feed intake, may suppress growth through reduction of serum IGF-I concentrations and increased expression of myostatin, thus providing evidence for mechanism (ii) listed above.

It could be hypothesized that the differences in growth response to PRRSV infection observed in our and previous studies may be due to differences in production of proinflammatory cytokines. This was investigated in a recent study (Petry et al., 2007), which compared expression levels of 11 immune genes involved in innate and adaptive immunity between pigs classified as high and low responders to PRRSV according to clinical and lesions symptoms from the HD and NEI lines used in their previous studies. The authors found that expression levels of IL-1β and IL-6 in the serum, lung, and bronchial lymph node were indeed greater for the leaner HD pigs than for pigs from the NEI line. However, line × treatment interactions were not statistically significant, thus providing no evidence for line differences in the production of these cytokines during infection.

Resource allocation has been considered as a main driver for the growth-disease susceptibility relationship by many authors (Beilharz et al., 1993; Rauw et al., 1998; Van der Waaij, 2004; Kyriazakis and Houdijk, 2008). The theory builds upon the assumption that different biological processes require nutritional resources. When these resources are scarce, a trade-off between different processes would occur, which is manifested by negative correlations between corresponding traits. Infectious challenges impose additional resource requirements for the animal (Lochmiller and Deerenberg, 2000), but may simultaneously trigger reduction in feed consumption (as illustrated for PRRSV infection above), thus producing a trade-off between growth and immunity. In particular, selection for lean growth may imply selection of animals that allocate consumed resources predominantly toward lean growth. When exposed to infectious challenge, these pigs may continue to allocate resources predominantly to growth at the early stages of the infectious challenge, thus not suffering great reductions in growth initially. However, neglecting the immune response in resource allocation may be detrimental for the immune status of the pig, leading to more severe disease symptoms and tissue damage, and, consequently, also to greater nutritional requirements to launch an effective immune response. Assuming that biological functions necessary for survival have priority over growth (Coop and Kyriazakis, 1999), the infected animal will eventually be forced to allocate a greater proportion of resources toward immunity with detrimental impact on growth. Hence, the theory infers that animals that have been bred to favor growth initially will suffer greater reduction in BW growth in the long run. This hypothesis was verified recently in a modeling study (Brindle, 2008). The results of our and previous studies (Petry et al., 2005) support the above theory, as line differences in reduction in growth were relatively small during the early stages of infection and disease symptoms were more severe for the pigs of the lines selected for lean growth at the acute phase of the selection, but differences in the symptoms decreased toward the later stage with detrimental impact on growth for pigs of the lean growth lines.

The above examples illustrate that the relationship between growth and disease state is likely to be the product of a variety of interacting mechanisms, which may be influenced differently by selection. A comprehensive framework that describes the interaction between the different mechanisms and the impact of selection on these would be desirable because it may help to anticipate the consequences of selection for PRRSV resistance. Conceptual frameworks for generic diseases have been proposed (Knap and Bishop, 2000; van der Waaij, 2004; Kyriazakis et al., 2008) and have also been implemented into a quantitative model for macro-parasitic infections (Vagenas et al., 2007; Doeschl-Wilson et al., 2008). To our knowledge, no such model exists for micro-parasitic infections. However, given the economic importance of PRRS and the vast amount of information produced by diverse experimental studies, PRRS appears to be a good candidate disease for the

development of a holistic model of the host-pathogen interaction.

LITERATURE CITED

Beilharz, R. G., B. G. Luxford, and J. L. Wilkinson. 1993. Quantitative genetics and evolution: Is our understanding of genetics sufficient to explain evolution? J. Anim. Breed. Genet. 110:161–170.

Blatteis, C. M. 2003. Fever: Pathological or physiological, injurious or beneficial? J. Therm. Biol. 28:1–13.

Brindle, W. 2008. Modelling the relationship between microparasitic infection, host nutrition, host genotype and growth. Master of Philosophy thesis. Edinburgh, UK.

Coligan, J. E., A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, and W. Strober, ed. 1996. Assessment of HIV titer using Reed-Muench accumulative method. In Current Protocols in Immunology. 3rd ed. Wiley, New York, NY.

Coop, R. L., and I. Kyriazakis. 1999. Nutrition-parasite interaction. Vet. Parasitol. 84:187–204.

Doeschl-Wilson, A. B., D. Vagenas, I. Kyriazakis, and S. C. Bishop. 2008. Challenging the assumptions underlying genetic variation in host nematode resistance. Genet. Sel. Evol. 40:241–264.

Escobar, J., W. G. Van Alstine, D. Baker, and R. W. Johnson. 2004. Decreased protein accretion in pigs with viral and bacterial pneumonia is associated with increased myostatin expression in muscle. J. Nutr. 134:3047–3053.

Galina-Pantoja, L., M. A. Mellencamp, J. Bastiaansen, R. Cabrera, G. I. Solano-Aguilar, and J. K. Lunney. 2006. Relationship between immune cell phenotypes and pig growth on a commercial farm. Anim. Biotechnol. 17:81–98.

Greiner, L. L., T. S. Stahly, and T. J. Stabel. 2000. Quantitative relationship of systemic virus concentration on growth and immune response in pigs. J. Anim. Sci. 78:2690–2695.

Halbur, P. G., P. S. Paul, M. L. Frey, J. Landgraf, K. Eernisse, X. J. Meng, M. A. Lum, J. J. Andrews, and J. A. Rathje. 1995. Comparison of the pathogenicity of two US porcine reproductive and respiratory syndrome virus isolates with that of the Lelystad virus. Vet. Pathol. 32:648–660.

Halbur, P. G., P. S. Paul, X. J. Meng, M. A. Lum, J. J. Andrews, and J. A. Rathje. 1996. Comparative pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a five-week-old caesarean-derived, colostrum-deprived pig model. J. Vet. Diagn. Invest. 8:11–20.

Halbur, P. G., M. F. Rothschild, B. J. Thacker, X. J. Meng, P. S. Paul, and J. D. Bruna. 1998. Differences in the susceptibility of Duroc, Hampshire, and Meishan pigs to infection with a high virulence strain (VR2385) of porcine reproductive and respiratory syndrome virus (PRRSV). J. Anim. Breed. Genet. 115:181–189.

Hart, B. L. 1988. Biological basis of the behaviour of sick animals. Neurosci. Biobehav. Rev. 12:123–137.

Johnson, W., M. Roof, E. Vaughn, J. Christopher-Hennings, C. R. Johnson, and M. P. Murtaugh. 2004. Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. Vet. Immunol. Immunopathol. 102:233–247.

Knap, P. W., and S. C. Bishop. 2000. Relations between genetic change and infectious disease in domestic livestock. Occas. Publ. Br. Soc. Anim. Sci. No. 27, BSAS, Edinburgh, Scotland, 65–80.

Kyriazakis, I., and J. C. M. Houdijk. 2008. Food intake and performance of pigs during health, disease and recovery. Pages 493–513 in Paradigms in Pig Science. J. Wiseman, M. A. Varley, S. McOrist, and B. Kemp, ed. Nottingham Univ. Press, Nottingham, UK.

Kyriazakis, I., F. B. Sandberg, and W. Brindle. 2008. Prediction of the consequences of pathogen challenges on the performance of growing pigs. Pages 398–418 in Mathematical Modelling in Animal Nutrition. J. France and E. Kebreab, ed. CABI, Wallingford, UK.

Labarque, G., S. Van Gucht, H. Nauwynck, K. van Reeth, and M. Pensaert. 2003. Apoptosis in the lungs of pigs infected with porcine reproductive and respiratory syndrome virus and associations with the production of apoptogenic cytokines. Vet. Res. 34:249–260.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos 88:87–98.

McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83–90.

Murtaugh, M. P., Z. Xiao, and F. Zuckermann. 2002. Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. Viral Immunol. 14:533–547.

Petry, D. B., J. W. Holl, J. S. Weber, A. R. Doster, and F. A. Osorio. 2005. Biological responses to porcine reproductive and respiratory syndrome virus in pigs of two genetic populations. J. Anim. Sci. 83:1494–1502.

Petry, D. B., J. Lunney, P. Boyd, D. Kuhar, E. Blankenship, and R. K. Johnson. 2007. Differential immunity in pigs with high and low responses to porcine reproductive and respiratory syndrome virus infection. J. Anim. Sci. 85:2075–2092.

Rauw, W. M., E. Kanis, E. N. Noordhuizen-Stassen, and F. J. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: A review. Livest. Prod. Sci. 56:15–33.

Roberts, N. E., and G. W. Almond. 2003. Infection of growing swine with porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*—Effects on growth, serum metabolites and insulin-like growth factor-I. Can. Vet. J. 44:31–37.

Sharma, M., R. Kambadur, K. G. Matthews, W. G. Somers, G. P. Devlin, J. V. Conaglen, P. J. Fowke, and J. J. Bass. 1999. Myostatin, a transforming growth factor-beta superfamily member, is expressed in heart muscle and is upregulated in cardiomyocytes after infarct. J. Cell. Physiol. 184:356–363.

Thanawongnuwech, R., B. J. Thacker, P. Halbur, and E. Thacker. 2004. Increased production of proinflammatory cytokines following infection with porcine reproductive and respiratory syndrome virus and *Mycoplasma hyopneumoniae*. Clin. Diagn. Lab. Immunol. 11:901–908.

Vagenas, D., A. B. Doeschl-Wilson, S. C. Bishop, and I. Kyriazakis. 2007. Exploration of the effects of host genotype and nutrition on the genetic parameters of lambs challenged with gastrointestinal parasites. Int. J. Parasitol. 37:1617–1630.

van der Waaij, E. H. 2004. A resource allocation model describing the consequences of artificial selection under metabolic stress. J. Anim. Sci. 82:973–981.

Van Reeth, K., G. Labarque, H. Nauwynck, and M. Pensaert. 1999. Differential production in proinflammatory cytokines in the pig lung during different respiratory virus infections: Correlations with pathogenicity. Res. Vet. Sci. 67:47–52.

Van Reeth, K., and H. Nauwynck. 2000. Proinflammatory cytokines and viral response disease in pigs. Vet. Res. 31:187–213.

Vincent, A. L., B. J. Thacker, P. G. Halbur, M. F. Rothschild, and E. L. Thacker. 2005. The in vitro susceptibility of macrophages to porcine reproductive and respiratory syndrome virus varies between two genetically diverse lines of pigs. Viral Immunol. 18:506–512.

Vincent, A. L., B. J. Thacker, P. G. Halbur, M. F. Rothschild, and E. L. Thacker. 2006. An investigation of susceptibility to porcine reproductive and respiratory syndrome virus between two genetically diverse commercial lines of pigs. J. Anim. Sci. 84:49–57.