

# PRRS Virus-Induced Damage to Intravascular Macrophages

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### Summary and Implications

The results of this research suggest: (1) PRRSV has a detrimental effect on the uptake of copper particles by pulmonary intravascular macrophages (PIMs), (2) the severity of PRRSV-induced damage to PIMs differs among PRRSV isolates, and (3) PRRSV-induced decreased pulmonary clearance supports the hypothesis that PRRSV infection makes pigs more susceptible to bacterial septicemia.

### Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-strand RNA virus recently classified in the family *Arteriviridae*. Antigenic, genetic, and pathogenic variation have been reported between U.S. and European isolates and among US isolates. Some herds are devastated by PRRSV-induced respiratory disease and secondary infections whereas other herds remain subclinical. PRRSV has been reported to replicate *in vitro* in pulmonary alveolar macrophages (PAMs) and more recently in PIMs<sup>4</sup>. We hypothesized that different isolates of PRRSV may have different effects on the uptake of copper particles by PIMs. In this study we compared low (RespPRRS<sup>®</sup> modified-live virus vaccine, NOBL Laboratories, Inc., Sioux Center, IA), and high (VR-2385) virulence strains of PRRSV in terms of pulmonary clearance of copper particles. Such findings could explain the association observed between PRRSV and secondary bacterial diseases in swine herds and help explain why certain PRRSV-infected herds have more problems with secondary bacterial infections.

### Materials and Methods

**Experimental design.** Seventy-five 3-week-old, crossbred, specific pathogen free, and PRRSV-free pigs were randomly assigned into three groups of 25 pigs each. The three treatments included intranasal inoculation with uninfected cell culture medium, RespPRRS<sup>®</sup> vaccine (low virulence group), or PRRSV isolate VR-2385 (high virulence group).

**Copper particle administration and copper analysis.** Five pigs from each group were necropsied at 3, 7, 10,

14, or 28 days post inoculation (DPI). Prior to necropsy, four of the five pigs from each group were given 0.2 ml/kg of 3% copper phthalocyanine tetrasulfonic acid diluted with normal saline (NS) to yield the total infusion volume of 2 ml/kg. The fifth pig in each group was given only NS so that baseline values of copper in sera and tissues could be established at each DPI. Pigs were euthanized with an intravenous overdose of sodium pentobarbital injection 30 minutes after the start of the infusion. To assess clearance of copper particles, samples of left caudal lung lobe, the left lobe of liver, spleen, and sera were analyzed for copper.

**Pathologic examination.** Complete necropsies were performed on all pigs. Sections for histopathologic examination were taken from lung, nasal turbinate, heart, brain, lymph nodes, tonsil, thymus, liver, and spleen. Immunohistochemistry was performed for detection of PRRSV antigens<sup>2</sup>.

**Virus isolation and serology.** The CRL11171 cell line was used to isolate PRRSV from bronchoalveolar lavage (BAL) fluid. Sera were obtained at 0, 3, 7, 10, 14, and 28 DPI.

### Results and Discussion

Gross lung lesions were scored as an estimated percent of the lung affected by grossly visible pneumonia. VR-2385 induced significantly more severe gross lung lesions ( $P < 0.05$ ). The percentage of the lung affected by pneumonia ranged from 0% to 5.6% in RespPRRS<sup>®</sup>-inoculated group and 15.2% to 46.4% in VR-2385-inoculated group with the peak lesions at 7 and 10 DPI, respectively. The microscopic lung lesions induced by VR-2385 were consistently more severe and diffuse than those in the RespPRRS<sup>®</sup> group. Lesions in the VR-2385 group persisted for 28 DPI, whereas the RespPRRS<sup>®</sup>-induced microscopic lung lesions had resolved by 14 DPI. PRRSV antigen was demonstrated in both PIMs and PAMs (data not shown) in RespPRRS<sup>®</sup>- and VR-2385-inoculated groups by immunohistochemistry.

PRRSV isolation was attempted only from the BAL fluid. PRRSV was recovered from the BAL fluid of 25 of 25 (100%) VR-2385-inoculated pigs, 13 of 25 (52%) RespPRRS<sup>®</sup>-inoculated pigs, and 0 of 25 uninoculated controls from 3 to 28 DPI.

We found that RespPRRS<sup>®</sup>-inoculated and uninfected control pigs had similar capacity to clear a single intravenous dose of copper particles, whereas the high virulence PRRSV VR-2385-inoculated pigs had significantly decreased copper concentrations in lungs correlated with decreased clearance of copper particles by PIMs at 7 to 14 DPI.

Decreased copper uptake by porcine PIMs also has been documented in fumonisin toxicity that induces ultrastructural alterations in PIMs resulting in an inability to remove blood-borne particles, thus increasing the susceptibility to bacteremic diseases<sup>3</sup>. Bertram<sup>1</sup> reported that porcine PIMs had an important role in the clearance of debris and *Actinobacillus pleuropneumoniae* (APP) from the blood. It is likely that PRRSV-induced damage to PIMs may result in increased susceptibility to bacterial diseases such as APP.

In conclusion, pulmonary clearance of intravenously administered copper particles is a useful and relatively simple method to measure one aspect of PIM function in pigs. PRRSV has an adverse effect on the uptake of copper particles by PIMs and the severity of this differs among PRRSV isolates. As expected, we have documented that the highly virulent VR-2385 isolate of PRRSV decreases the ability of PIMs to clear copper particles, thereby potentially increasing susceptibility to bacteremic disease. Although copper uptake by PIMs differs somewhat from bacterial endocytosis, these results could explain the increased in the chronic respiratory diseases and death loss experienced in pigs on farms endemically infected with high virulence strains of PRRSV.

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