# Sponsors

**University of Minnesota**
College of Veterinary Medicine

College of Agricultural, Food and Environmental Sciences

Extension Service

Swine Center

## Editors
W. Christopher Scruton
Stephen Claas

## Layout
David Brown

## Logo Design
Ruth Cronje, and Jan Swanson;
based on the original design by Dr. Robert Dunlop

## Cover Design
Sarah Summerbell

# Virological and Immunological Parameters of the Acute Phase of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Infection

[2]M. Olin*, [3]Z. Xiao [1]L. Batista [1]S.A. Dee [1]C. Pijoan, , [3]M. Murtaugh, [2]T.W. Molitor [1]SDEC, [2]CAPS, [3]Veterinary Pathobiology University of Minnesota, St. Paul, MN, 55108.

## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) constitutes one of the most important disease problems that the swine industry faces today. PRRSV is an RNA virus classified in the *Arteriviridae family,* order *Nidovirales*. Swine develop both humoral and cell-mediated immunity after PRRSV infection but their relative importance in protection and clearance of the virus is not yet completely understood. PRRS convalescent animals show specific protective antibodies (IgM and IgG) developing 7 to 14 days post infection (1) detectable by indirect fluorescent antibody test (IFA), serum virus neutralization test (SN), immunoperoxidase monolayer assay (IPMA) and enzyme-linked immunosorbent assay (ELISA). Furthermore, an antigen-specific T-cell response to PRRS field virus inducing a long lasting CMI response in pigs has been shown (2). Virus-specific IFN-γ-secreting T cells and virus neutralizing antibodies which have the ability to eliminate the virus (3) have not been detected until several weeks after exposure to either wild-type or vaccine virus (4). Likewise, levels of T cell secreting IFN-γ relate to protective immunity. The identification of key immune parameters and the basis for this apparent significant delay in the establishment of protective immunity in the host following PRRSV infection may lead to the development of diagnostic assays that could distinguish persistent carriers from animals that have cleared the virus.

## Objectives

Within a large population of experimentally infected female swine during the first 30 days post infection, the following objectives will be performed: 1. To assess the kinetics of PRRSV persistence in the host during the acute phase of infection. 2. To characterize the kinetics of cellular immune response of different lymphocyte populations 3. To investigate the cellular response to PRRSV of lymphocytes from different tissues.

## Materials and Methods

Eighty, 4-month old female swine originating from a PRRSV-naïve source were inoculated on day 0 with the field isolate MN-30100 at a concentration of $10^{2.4}$ $TCID_{50}$ (total dose). Fifteen animals were bled on days 0, 3, 7, 14, 21 and 30. On day 30, ten pigs were sacrificed and peripheral blood mononuclear cells (PBMNC), as well as the following lymphoid tissues were collected: sternal lymph node (SLN), mesenteric lymph node (MLN), inguinal lymph node (ILN), spleen and bone marrow (BM). The cell-mediated immune response to interferon-gamma levels were analyzed by the standard Elispot assay, and intracellular and surface staining by Flow Cytometry. Sera was tested for the presence of PRRSV antibodies by ELISA, for PRRSV nucleic acid by polymerase chain reaction (PCR), and for viable PRRSV by virus isolation (VI). Specifically, the IDEXX ELISA (IDEXX Laboratories Westbrook, ME) and the Taqman ™ PCR (Perkin-Elmer Applied Biosystems, Foster City, CA) were used, and samples were assessed for viable virus using MARC-145 continuous cell lines and porcine alveolar macrophages. On Day 0, three 8-week old PRRSV naïve gilts originating from the same source were housed in a separate facility, 30 m from the experimental facility and served as negative controls to monitor lateral introduction of PRRSV.

## Results

All 15 index gilts from the monitoring group were PRRSV-negative on arrival, as verified by PCR, VI, and ELISA. Serial testing of the monitoring gilts indicated successful experimental infection. On day 3 pi, 15/15 of the tested gilts were PCR positive and 0/15 were VI positive. Clinically, animals were depressed, anorexic and feverish (40 to 41.5 $^{0}$ C) for approximately 48 to 72 hours pi. No mortality was observed. Molecular sequencing of open reading frame (ORF 5) a randomly selected PRRSV isolate recovered from an index pig indicated 100% homology with the isolate used for the experimental infection. On day 7 pi, 15/15 of the gilts in the monitoring group were PCR positive and 8/15 were VI positive; however, all were ELISA negative (sample-to-positive ratio < 0.4). On day 14 pi, 15/15 gilts in the monitoring group were ELISA positive, while 15/15 and 9/15 were PCR and VI positive, respectively. On day 21 pi, 15/15 were ELISA positive, 14/15 PCR positive and 0/15 were positive by VI. The number of ELISA positive monitoring gilts detected on day 30 was 15/15. Only one serum sample collected on day 30 was PCR positive and 15/15 VI negative. Also on day 30 all the pooled

tissues from the 10 sacrificed animals were PCR positive.

Phenotyping of γδ and αβ lymphocytes indicated that from day 0 to 14 there were no significant changes in the different cell populations From day 15 to day 30, the population of circulating γδ lymphocyte increased from 22 to 40% while the CD8 population decreased from 33 to 21%. CD4 and dual positives remained unchanged. Antigen stimulated interferon-γ levels analyzed by flow cytometry showed a significant increase of interferon-γ by γδ T cells compared to the CD4 T cells between days 14 and 30 pi. In PRRSV specific interferon-γ Elispot, the number of positive cells increased over time. On day 30, the majority of sampled pigs had higher positive cell numbers compared to those on day 14. Antigen specific blastogenesis analyzed by flow cytometry showed a significant increase between γδ T cells compared to the CD4 T cells between days 14 and 30. On day 30, lymphocytes collected from PBMC, spleen and ILN were similar in number of PRRSV stimulated interferon-γ positive spots analyzed by Elispot. However, BM showed the highest positive number of positive spots.

## Discussion

Diagnostic data from the monitoring gilt group indicated detectable viremia in 10% of the index population on day 30 pi. PRRSV RNA was detected by PCR in all 10 gilts sacrificed on day 30. PRRSV-specific cell mediated responses were demonstrated by the presence of interferon-γ and lymphocyte blastogenesis after on 14 and day 30 pi. γδ T cells response to antigen stimulation was higher compared to CD4 T cells. These data differ from previously published results, which reported more delayed interferon-γ levels post infection. The reasons for these differences may be due to sample size, age of the pigs, PRRSV strain, or experimental design.

## References


1. Allende R et al (2000) Journal of Virology 74(22): 10834-10837.
2. Meier et al. (2000) Veterinary Research 31:41.
3. Osorio F (2002) AASV 361-365.
4. Zuckermann FA et al. (1999) Adv Vet Med. 41: 447-61.