

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION: USDA, ARS, BARC

A. NC-229 REPRESENTATIVE:

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Other PRINCIPLE LEADERS associated with the projects

Wysocki, Michal; Chen, Hongbo; BARC

Rowland, R.R.R., KSU

Zimmerman, Jeff; Molina, Ramon; Reecy, Jim; Rothschild, Max; Iowa State Univ. (ISU)

Christopher-Hennings, Jane; Fang, Ying; Nelson, Eric A.; South Dakota State Univ. (SDSU)

McCaw, Monte B. (deceased), North Carolina State Univ. (NCSU)

Smith, Doug; Ho, Sam; Univ. MI

Steibel, JP; Ernst, Cathy; Michigan State Univ. (MSU)

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Elucidate the mechanisms of host-pathogen(s) interactions.

The porcine reproductive and respiratory syndrome (PRRS) Host Genetics Consortium (PHGC) was developed to determine the role of host genetics in resistance to PRRS and in effects on pig health and related growth effects. The PHGC is a multi-year project that is funded by a US consortium representing the US National Pork Board (NPB), USDA, universities and private companies; it represents the first-of-its-kind approach to food animal infectious disease research. The project uses a Nursery Pig Model to assess pig resistance/ susceptibility to primary PRRSV infection. Crossbred pigs from high health farms were donated by commercial sources and transported at weaning to the biosecure Kansas State University facilities. After acclimation, the pigs were infected with PRRSV and followed for 42 days post infection (dpi). Blood samples were collected at 0,4,7,10,14,21,28,35 and 42 dpi and weekly weights recorded. DNA from all PHGC pigs has been prepared and is being genotyped with the PorcineSNP60 Genotyping BeadChip (containing over 60K single nucleotide polymorphisms or SNPs). Data is being collated in the PHGC relational database at ISU. Results from the first 5 trials of 200 pigs each have affirmed that all pigs become PRRSV infected; some pigs clear virus from serum quicker and weight effects are variable. Multivariate analyses of viral load and weight data have identified PHGC pigs in different virus/weight categories, so that ongoing serum cytokine and gene expression studies can compare data from PRRS resistant/maximal growth pigs to PRRS susceptible/reduced growth pigs. The USDA PRRS Coordinated Agricultural Project (PRRS CAP) and NRSP8 Swine Genome Coordinator are supporting the state-of-the-art whole genome association and SNP chip analyses to identify genetic determinants of resistance/susceptibility. Overall, the PHGC project will enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection.

Identifying host gene expression changes that are involved in regulating responses to PRRSV infection and vaccination. With samples collected at NCSU BARC scientists are testing the effect of PRRSV infection or vaccination on pigs using RNA prepared from tracheobronchial lymph nodes (TBLN), the cranial and distal part of the lung, and tonsils. Pigs were either infected with Minnesota (MNW2B) or NC Powell strains of PRRSV or vaccinated with ATP or were non-treated controls. Mucosal tissue samples were collected from pigs between 3 and 6 days post treatment so that the early innate immune response could be evaluated. RNAs were prepared and hybridization to the swine long oligo array [pigoligoarray.org Steibel et al. 2009] could be assessed. Analyses are underway at BARC with statistical assessment of gene expression patterns performed in collaboration with MSU scientists. Tests of the effect of samples collected after homologous or heterologous PRRS vaccination and challenge are also underway.

Verifying the role of the nonstructural protein 2 (nsp2) of PRRSV in viral replication and modulation of host immunity. Work at SDSU identified six immunodominant nsp2 B-cell epitopes (ES2 through ES7) that were deleted from a Type I PRRSV cDNA infectious clone. Deletion of ES3, ES4, or ES7 allowed the generation of viable virus. The Δ ES3 mutant showed increased cytolytic activity and more vigorous growth kinetics, while Δ ES4 and Δ ES7 mutants displayed decreased cytolytic activity and slower growth kinetics in vitro. In a nursery pig model, Δ ES4 and Δ ES7 mutants exhibited attenuated phenotypes and the Δ ES3 mutant produced higher peak viral loads. Interleukin-1 beta (IL-1 β) and TNF- α expression levels were down-regulated in cells stimulated (or infected) with the Δ ES3 mutant. These results suggest that certain regions in nsp2 are non-essential for PRRSV replication and may play an important role in modulation of host immunity in vivo.

Objective 2. Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine.

None

Objective 3. Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine.

In collaboration with SDSU a multiplex assay is being developed to simultaneously quantify 9 porcine cytokines in serum using Luminex xMap™ technology was developed and optimized to detect innate [interleukin-1beta (IL-1b), IL-6, IL-8, interferon-alpha (IFN-a), TNF-a]; regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN-gamma) and Th2 (IL-4) cytokines. The assay will be of value in vaccine and challenge studies as well as for determining genetic resistance to PRRSV and immune responses to other swine pathogens.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

The PRRS Host Genetics Consortium (PHGC) has begun to determine the role of host genetics in resistance to PRRS and in effects on pig health and related growth effects. Using a Nursery Pig Model crossbred pigs from high health farms were infected with PRRSV and followed for 42 days. Results from the first 5 trials of 200 pigs each have affirmed that all pigs become PRRSV infected but pigs clear virus from serum at different rates; weight effects are variable. Overall,

the PHGC project will enable researchers to verify important genotypes and phenotypes that predict resistance/susceptibility to PRRSV infection.

A multiplex assay to simultaneously quantify 9 porcine cytokines in serum using Luminex xMap™ technology was developed and optimized to detect innate (IL-1b, IL-6, IL-8, IFN- α , TNF- α); regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN- γ) and Th2 (IL-4) cytokines. The assay will be of value in vaccine and challenge studies as well as for determining genetic resistance to PRRSV and immune responses to other swine pathogens.

The PRRSV nsp1 protein was determined to antagonize beta interferon (IFN- β) responses. We demonstrated that nsp1 β inhibits both interferon synthesis and signaling, while nsp1 α alone strongly inhibits the synthesis of interferon. These findings provide important insights into the mechanisms of how nsp1 contributes to PRRSV pathogenesis and how this may impact future vaccine development strategies.

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

- Ando A, Uenishi H, Kawata H, Tanaka M, Shigenari A, Flori L, Chardon P, Lunney JK, Kulski JK, Inoko H. 2008. Microsatellite diversity and crossover regions within homozygous and heterozygous SLA haplotypes of different pig breeds. *Immunogenetics*. 60: 399-407.
- Opriessnig T, Madson DM, Prickett JR, Kuhar D, Lunney JK, Elsener J, Halbur PG. 2008. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. *Vet. Microbiol*. 131: 103-14.
- Lunney JK. 2008. Genetics of Infectious Disease Resistance in Animals. *Proceedings American College of Veterinary Pathologists Meeting 2008*. P. 240-242.
- Tuggle CK, Wang YF, Couture OP, Qu L, Uthe JJ, Kuhar D, Lunney JK, D. Nettleton D, J.C. Dekkers JC, Bearson SMD. 2008. Computational Integration of Structural and Functional Genomics Data across Species to Develop Information on Porcine Inflammatory Gene Regulatory Pathway. *Dev Biol (Basel)*. 132: 105-13.
- Lunney JK, Ho C-S, Wysocki M, Smith DM. 2009. Molecular genetics of the swine major histocompatibility complex, the SLA complex. *Dev Comp Immunol*. 33: 362-74.
- Ho C-S, Lunney JK, Franzo-Romain MH, Martens GW, Lee Y-J, Lee J-H, Wysocki M, Rowland RRR, Smith DM. 2009. Molecular characterization of swine leukocyte antigen (SLA) class I genes in outbred pig populations. *Animal Genetics*. 40: 468-78.
- Ho C-S, Lunney JK, Ando A, Rogel-Gaillard C, Lee J-H, Schook LB, Smith DM. 2009. Nomenclature for factors of the SLA system, update 2008. *Tissue Antigens*. 73: 307-315.
- Opriessnig T, Patterson AR, Madson DM, PalM, Rothschild M, Kuhar D, Lunney JK, Juhan NM, Meng XJ, Halbur PG. 2009. Difference in severity of porcine circovirus type 2 (PCV2)-induced pathological lesions and disease between Landrace and Pietrain pigs. *J. Animal Science*. 87: 1582-90.
- Steibel JP, Wysocki M, Lunney JK, Ramos AM, Hu Z-L, Rothschild MF, Ernst CW. 2009. Validation of the Swine Protein-Annotated Oligonucleotide Microarray. *Animal Genetics*. 40: 883-893.

- Ho C-S, Lunney JK, Lee J-H, Franzo-Romain MH, Martens GW, Rowland RRR, Smith DM. 2009. Molecular characterization of swine leukocyte antigen (SLA) class II genes in outbred pig populations. *Animal Genetics*. In Press.
- Chen Z, Zhou X, Lunney JK, Lawson S, Sun Z, Brown E, Christopher-Hennings J, Knudsen D, Nelson EA, Fang Y. 2009. Immunodominant epitopes in nsp2 of porcine reproductive and respiratory syndrome virus are dispensable for replication but play an important role in viral pathogenesis. *J Gen Virol*. Epub. 11/18/09.
- Lunney JK, Fritz ER, Reecy JM, Kuhar D, Prucnal E, Molina R, Christopher-Hennings J, Zimmerman J, Rowland RRR. 2009. Interleukin-8, interleukin-1 β and interferon- γ levels are linked to PRRS virus clearance." *Viral Immunology*. In Revision.
- Boyd P, Hudgens E, Loftus JP, Tompkins D, Wysocki M, Kakach L, LaBresh J, Baldwin CL, Lunney JK. 2009. Expressed gene sequence and bioactivity of the IFN γ -response chemokine CXCL11 of swine and cattle. *Vet. Immunol. Immunopathol*. Submitted.

2) Abstracts or Proceedings

- Chen Z, X Zhou, D Kuhar, S Lawson, J Lunney, Y Fang. 2008. Effect of PRRSV nsp2 epitope deletion mutants on the induction of cytokine response in porcine alveolar macrophages. 2008 CRWAD & 2008 PRRSV Symp.
- Fritz E, Hu Z, Lunney J, Reecy J. 2008. The PHGC Database: management of large data sets. Intl PRRS Symp. #324. 12/08
- Lawson S, Lunney JK, Fang Y, Nelson EA, Christopher-Hennings J. 2009. Development of a rapid, swine-specific microsphere assay to simultaneously detect multiple immune proteins (cytokines) affected by porcine reproductive and respiratory syndrome virus (PRRSV) infection. 2009 Intl PRRS Symp. and CRWAD 12/09.
- Lunney JK. 2008. Genetics of Infectious Disease Resistance in Animals: Pig and PRRS. Proc. Am Coll Vet Pathol. Meeting. 11/08
- Lunney JK. 2009. PRRS Host Genetics Consortium: Current Progress and Potential for Canadian Involvement. Canadian Centre for Swine Improvement meeting, Quebec City, Canada 6/09
- Lunney JK, Boyd P, LaBresh J, Kakach L, Wagner B, Tompkins D, Hudgens E, Baldwin C. 2009. Swine Toolkit progress for the US Veterinary Immune Reagent Network. 2009 Intl PRRS Symp. and CRWAD 12/09.
- Lunney JK, Boyd P, Prucnal L, Zarlenga D, LaBresh J, Steffens C, Wagner B, Tompkins D, Hudgens T, C Baldwin C. 2008. Swine Toolkit progress for the US Veterinary Immune Reagent Network. Intl PRRS Symp. #288 and CRWAD 95P. 12/08
- Lunney JK, Reecy J, Rowland RRR. 2009. PRRS Host Genetics Consortium: Current Progress and Potential for Canadian Involvement. Canadian Swine Health Forum 2009: July 7-8, 2009, Saskatoon, SK, Canada.
- Lunney JK, Reecy J, Rowland RRR. 2009. Current Progress of US PRRS Host Genetics Consortium. Genomics for Animal Health: Outlook for the Future (EADGENE 2009) meeting, 10/09, Paris, France.
- Lunney JK, Rowland RRR, Chen Z, Zhou X, Lawson S, Sun Z, E. Brown E, J. Christopher-Hennings J, Nelson E, Fang Y. 2009. Genetic approaches to reveal immune response pathways and viral antigen targets for novel vaccine design. Intl Vet Vaccines and Diagnostics Conference (IVVDC 2009), WI. 7/09.

- Lunney JK, Steibel JP, Reecy J, Rothschild M, Kerrigan M, Tribble B, Rowland RRR. 2009. PRRS Host Genetics Consortium: Current Progress. 2009 Intl PRRS Symp. and CRWAD 12/09.
- Lunney JK, Wysocki M, Steibel JP, Kuhar D, Ernst CW, McCaw M. 2009. Uncovering Genetic Components Involved In Regulating Early Immune Responses To Porcine Reproductive And Respiratory Syndrome (PRRS). PAG2009. PAG-XVII P640. 1/09
- Rowland RRR, Kerrigan M, Bujuru S, Tribble B, Lunney JK. 2009. An infection model for the study of PRRS at the population level. 2009 Intl PRRS Symp. 12/09.
- Tuggle CK, Bearson SMD, Uthe JJ, Christian C, Couture O, Demirkale CY, Nettleton D, Lunney JK, Honavar V. 2009. Using transcriptomic data to develop tools for predicting shedding traits in growing pigs. CRWAD 12/09.
- Wong SJ, Lunney JK, Rowland RRR. 2009. Nucleocapsid protein-specific IgG and IgM responses in oral fluids during PRRSV infection. 2009 Intl PRRS Symp. 12/09.
- Wysocki M, SteibelJP, McCaw M, Kuhar D, Ernst CW, Lunney JK. 2008. Uncovering genetic components involved in early regulatory immune response during PRRSV infection. Intl PRRS Symp. #285 and CRWAD #125. 12/08

3) Book chapters or monographs

- Santos I, Lunney JK, Ferreira B (CoEditors). 2009. Special Issue: Proceedings of 8th International Veterinary Immunology Symposium, Brazil. Vet. Immunol. Immunopathol. 128 (1-3): 1-289.

E. FUNDING SOURCES FOR PRRSV RESEARCH

1) Current

- Rowland RRR, JK Lunney, R Johnson, J Reecy. PRRS Host Genetics Consortium: A proposal to develop a consortium to study the role of host genetics and resistance to PRRSV. National Pork Board #07-233 Animal Health and Animal Science \$300,000, 2007-2008; Renewal \$250,000 2009-2010.
- Fang Y, JK Lunney, J Christopher-Hennings, E Nelson, A Young. The role of PRRSV non-structural proteins 1 and 2 in host immunity. USDA-NRI, \$375,000, 1/08-12/2010.
- Christopher-Hennings, J, Y Fang, J Lunney, EA Nelson. Development of a rapid, single tube, multiplex test to simultaneously detect immune parameters (cytokines) induced by PRRSV. National Pork Board, \$101,107. 2008-2009
- Lunney JK, J Dekkers, R Fernando, Z Jiang, H-C Liu, R Pogranichniy, JM Reecy, R Rekaya, M Rothschild, D Smith, JP Steibel, C Tuggle. PRRS CAP Host genetics: Characterization of host factors that contribute to PRRS disease resistance and susceptibility. USDA NIFA PRRS CAP2: Objective 3 Host Genetics. \$560,000. 2009-2012.
- JK Lunney, C Ernst, V. Honavar, Z Jiang, R Pogranichniy, JP Steibel, C Tuggle. Identifying porcine genes and gene networks involved in effective response to PRRS virus using functional genomics and systems biology. USDA AFRI/NIFA Animal Genome, Genetics, and Breeding Program. \$750,000. 2010-2012
- Fang Y, JJ Zimmerman, J Christopher-Hennings, EA Nelson, M Murtaugh, JK Lunney. Development of diagnostic assays for detecting PRRSV infection using oral fluid samples as an alternative to serum-based assays. National Pork Board. \$119,960.

Lunney JK, J Christopher-Hennings, EA Nelson, Y Fang, JP Steibel, J Zimmerman. Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay. National Pork Board. \$103,929.

F. WORK PLANNED FOR NEXT YEAR

New assays related to PRRSV research priorities will be developed and optimized, including assays using the BioRad Bio-Plex platform. Further evaluation and collaborative utilization of swine cytokine multiplex assays and development of new infectious agent detection assays for oral fluids and other substrates will be priorities. Additionally, we will continue to develop and distribute “shared resources” to the research community, including antibodies, virus isolates, sequences and protocols.