

## ANNUAL REPORT PROJECT NC-229

**PERIOD COVERED:**        **October 2008 to June 2009**

**INSTITUTION OR STATION:** **OARDC, The Ohio State University, Ohio**

**A. NC-229 REPRESENTATIVE:**

**Gourapura, Renukaradhya J.**, Food Animal Health Research Program (FAHRP), Ohio Agricultural Research and Development Center (OARDC), The Ohio State University, [gourapura.1@osu.edu](mailto:gourapura.1@osu.edu)

**Other PRINCIPLE LEADERS associated with the projects**

Saif, Linda J. FAHRP, OARDC, The Ohio State University.

Benfield, David A. Associate Director, OARDC, The Ohio State University.

**B.        PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:**

***Objective 1. Evaluation of mucosal immune responses in the respiratory tract of pigs using innate immune cell specific agents as candidate adjuvants administered by the intranasal (IN) route.***

A new laboratory to study mucosal immune responses to PRRSV and development of novel mucosal vaccines to control PRRS outbreaks has been set up in FAHRP, OARDC, The Ohio State University, at Wooster, Ohio. Collaborators included, Drs. Eric A Nelson, South Dakota State University, Michael Murtaugh, University of Minnesota, Larry Schlesinger, Director of Center for Microbial Interface Biology, The Ohio State University, Michael Roof, Executive Director of Bio-R&D, Boehringer Ingelheim Vetmedica Inc., and Colorado University, TB vaccine testing and research material facility.

Currently, PRRS-MLV vaccine administered *systemically* protects against homologous PRRSV challenge, but failed to protect against field viral re-infections, as well as infections caused by heterologous PRRSV strains. Effective and specific anti-viral mucosal immune responses prevent entry of pathogens into the body, and protect against homologous and against heterologous viral challenge. Mucous membranes cover the largest surface area in the body. The mucous-associated-lymphoid tissues contain more than 80% of the immune cells to prevent entry of pathogens into the body, and also to maintain immune homeostasis. Stimulating the immune system systemically (i.e. by injections) results in systemic protection, but with only low mucosal immunity. However, direct stimulation of the mucosal immune system results in production of both mucosal (local) and systemic protection, so that infectious agents are blocked from entry into the body. Despite these benefits, in practice it has often proven difficult to generate strong protective mucosal immunity to pathogens, due to dominant immune regulatory mechanisms at mucosal surfaces. Therefore, to generate protective anti-microbial mucosal immunity, suitable adjuvants/delivery systems to circumvent the mucosal regulatory barrier are needed. The aim of our current project was to identify appropriate candidate adjuvants which could help to elicit protective mucosal immunity to PRRSV at the mucosal surfaces of the pig respiratory tract. We initially determined the adjuvanticity of nine different bacterial preparations belongs to *Mycobacterium tuberculosis*, *Streptococcus pyogenes*, and *Vibrio cholera*, administered

intranasally (IN) to pigs. Based on the general mucosal immune responses elicited by the individual candidate adjuvants, we chose *M. tuberculosis* whole cell lysate (*M. tb* WCL) for further studies. Analyses are underway to explore the extended adjuvanticity of *M. tb* WCL, used with PRRSV modified live virus vaccine (Boehringer Ingelheim Vetmedica Inc.,) administered IN to PRRSV sero-negative pigs. Also we will perform challenge studies in mucosally immunized pigs using homologous (VR2332) and heterologous (MN184) PRRSV strains.

***Objective 2. Natural killer (NK) cell-mediated innate immune cytotoxicity in PRRSV and porcine respiratory coronavirus (PRCV) infected pigs.***

In collaboration with Dr. L.J Saif's laboratory, we evaluated the innate and adaptive immune responses in pigs infected with PRRSV, and then 10 days later co-infected with PRCV. The PRRSV/PRCV dual virus-infected pigs had significantly suppressed innate immune responses, as evidenced by reduced interferon (IFN)- $\alpha$  level in lung and blood. In addition, we identified a significant reduction in systemic NK cell-mediated cytotoxicity in PRRSV alone infected pigs. Further, upon co-infection with PRCV, there was a synergistic suppression of NK cell-mediated cytotoxicity. Co-infection by PRRSV and PRCV led to enhanced PRRSV replication in lung and a trend towards increased serum Th1 (IFN $\gamma$  and IL-12), but decreased Th2 (IL-4) cytokine responses, thus clinically exacerbating PRRSV pneumonia. These findings imply that a prior innate immune suppression by immunomodulating respiratory viruses (like that induced by PRRSV) may be a contributing factor to more severe pneumonia due to respiratory coronavirus infection.

**C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:**

We have identified the potential use of bacterial preparations as candidate adjuvants to augment anti-PRRSV mucosal immune responses in pigs. Our study identified one more innate immune suppressive function of PRRSV in pigs, mediated through NK cells. As an essential component of PRRS control and prevention, we have made significant progress in the area of mucosal vaccine production and in understanding innate immune responses to PRRSV. NPB funded our study on development of mucosal vaccine to PRRSV to RG. PRRSV and PRCV co-infection study was funded by National Institute of Health to LJS.

**D. PRRS PUBLICATIONS ISSUED OR "IN PRESS"**

**1) Refereed publications**

Jung, K., **G.J. Renukaradhya**, K.P. Alekseev, Y. Fang, Y. Tang, and L.J. Saif (2009). Porcine reproductive and respiratory syndrome virus modifies innate immunity and alters disease outcome in pigs subsequently infected with porcine respiratory coronavirus: implications for respiratory viral co-infections. *J. Gen. Virol.* 90:2713-23.

**Renukaradhya, G.J.**, K.P. Alekseev, K. Jung, Y. Tang, Y. Fang, and L.J. Saif (2009). Altered immune responses to porcine respiratory coronavirus in pigs previously infected with porcine reproductive and respiratory syndrome virus. *Vet. Immunol. Immunopath.* (In review).

**2) Abstracts or Proceedings**

K. Jung, K. Alekseev, **G.J. Renukaradhya**, Y. Tang, Y. Fang, P. Lewis, X. Zhang, L.J. Saif. Altered pathogenesis of porcine respiratory coronavirus (PRCV) in the presence of PRRSV infection and their pathologic relationships: Potential effect of preexisting respiratory viral

infections on SARS severity. XIV International Congress of Virology, American Society of Virology, Annual Meeting at Cornell University, Ithaca, NY, 11-15 August 2008.

- K. Jung, K. Alekseev, **G.J. Renukaradhya**, Y. Tang, Y. Fang, P. Lewis, X. Zhang, L.J. Saif. Altered pathogenesis of porcine respiratory coronavirus (PRCV) subsequent to PRRSV infection: Model for effect of respiratory viral co-infections on SARS severity. CRWAD Meeting, Chicago, IL, December 7- 9, 2008.
- G. J. Renukaradhya**, Konstantin Alekseev, Kwonil Jung, and Linda J. Saif. Distorted immune responses in pigs to porcine respiratory coronavirus previously infected with porcine reproductive and respiratory syndrome virus: a respiratory viral co-infection model. CRWAD Meeting, Chicago, IL, December 6- 8, 2009.
- V. Dwivedi, C. Manickam, R. Patterson, K. Dodson, and **G. J. Renukaradhya**. Steps towards development of a novel mucosal vaccine to PRRSV. Fourth International Scholar Research Exposition, The Ohio State University, November 19<sup>th</sup>, 2009
- V. Dwivedi, C. Manickam, R. Patterson, K. Dodson, and **G. J. Renukaradhya**. Development of a novel mucosal vaccine to protect against porcine reproductive and respiratory syndrome in pigs. 2009 PRRSV Symposium.

## **E. FUNDING SOURCES FOR PRRSV RESEARCH**

### **1) Current**

- Renukaradhya J. Gourapura. Evaluation of adjuvants at the mucosal area for the development of innovative mucosal vaccine against PRRS. National Pork Board. Nov. 2008 to May 1, 2010.
- Renukaradhya J. Gourapura. Study of mucosal immune responses in the respiratory tract of pigs infected with porcine reproductive and respiratory syndrome virus. OARDC Seed grant, The Ohio State University. March 2009 to February 2011.
- Michael Murtaugh and Renukaradhya J. Gourapura. Positive Prognosticators of Immune Protection and Prophylaxis against PRRSV in Swine Herds. PRRSV PRRS CAP 2. August 2009 to July 2013.
- Renukaradhya J. Gourapura. Development of novel mucosal vaccines for the control of PRRSV outbreaks. National Pork Board. Dec. 2009 to May, 2011.

## **F. WORK PLANNED FOR NEXT YEAR**

We will complete our ongoing project on evaluation of adjuvants at the mucosal area for the development of innovative mucosal vaccine against PRRS. We will start our new project to evaluate remaining two bacterial candidate adjuvants which have the potential to elicit protective mucosal immunity to PRRSV. We will also begin our studies on development of PLGA microspheres based killed PRRSV vaccine trials. In collaboration with Dr. Murtaugh we will begin our research to characterize the effect of PRRSV vaccine intervention on innate immune parameters in naïve finishing herd to PRRSV outbreaks (an objective of PRRS CAP2).