

## ANNUAL REPORT PROJECT NC-229

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

**INSTITUTION OR STATION:**

**A. NC-229 REPRESENTATIVE:**

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**Other PRINCIPLE LEADERS associated with the projects**

Tripp, Ralph; UGA

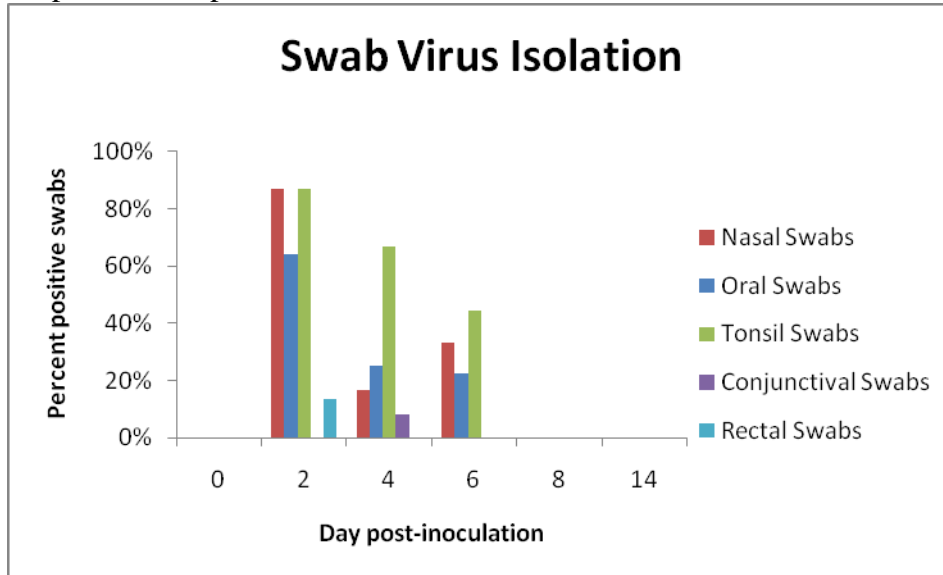
**B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:**

*Limited progress was made at UGA on PRRSV-related projects.* Investigators intended to continue development of novel vaccine strategies for prevention of PRRSV infection of swine, however, all efforts were set aside because of the outbreak and ultimate influenza pandemic caused by the swine-origin H1N1 virus. Below is a summary of this work and future plans to address PRRSV, as well.

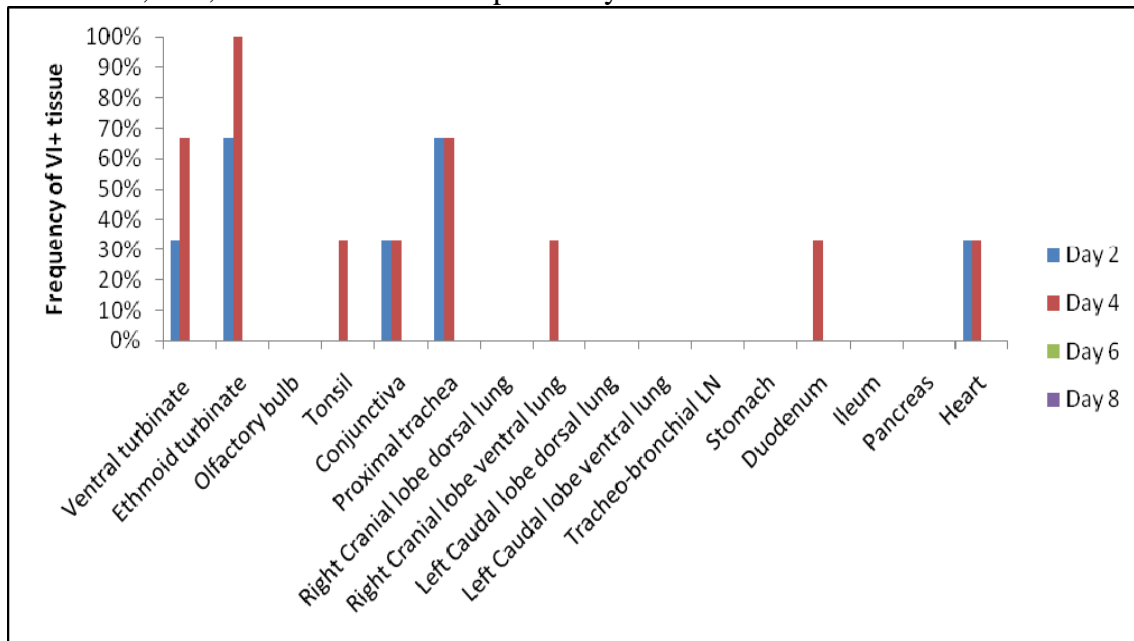
The novel, swine-origin H1N1 (pandH1N1) virus was first identified in April 2009. UGA received the virus (A/CA/04/09) in June 2009 and initiated pathogenesis studies in swine and ferrets. The ferret serves as the primary model of human influenza virus infection. It is susceptible to infection with human influenza viruses without adaptation, sheds virus, and presents similar symptoms. Remarkably, the pandH1N1 replicated to very high titers in ferrets (>1e8 TCID<sub>50</sub>/ml of nasal wash), even when inoculated with very low 100 TCID<sub>50</sub>) doses of virus but failed to demonstrate significant clinical disease. Virus primarily replicated in the upper respiratory tract, but in some cases was detected in the lungs of infected ferrets.

The swine studies have been much more extensive. The objective was to test the emergent pandemic (H1N1) 2009 influenza (pandH1N1) virus for infectivity and pathogenicity in the swine model of influenza virus infection. Develop the swine animal model for influenza virus infection, transmission, reassortment, and pathogenesis. At the time studies were initiated, BSL2 containment with BSL3 practices was recommended for laboratory studies, however CDC did not provide recommendations for animal work. USDA recommended ABSL3 containment and practices for pandH1N1 infection in agricultural species and asked that we have a modified Import or Transport Controlled Material or Organisms or Vectors (VS 16-3) permit from APHIS to receive pandH1N1 for use in ferrets and poultry in addition to existing VS 16-3 permits for swine influenza. UGA received USDA approval for the studies on in June and infection, pathogenesis, and shedding studies were initiated later in the summer. Influenza seronegative (pandH1N1, swH1N1) were inoculated with 1e6 TCID<sub>50</sub> of a C1/C2 passage of A/CA/04/09 intranasally in 1.0ml (0.5ml / nostril). Animals were monitored daily. Serum, whole blood,

swabs and environmental samples were collected every other day after challenge through day 8 and on day 14. Also, 3 animals were euthanized on days 2, 4, 6, 8, and 14 for tissue virus titration and pathology. This large study generated over 1200 serum, blood, tissue, and swab samples for analysis. In brief, A/CA/04/09 infected pigs and caused clinical symptoms including loss of appetite, coughing, nasal discharge, and reduced activity. Dr. Howerth is currently scoring and compiling all hisotpathological results. Serology is being completed. Virus isolation endpoints are reported below.



Swine were inoculated with 10e6 TCID<sub>50</sub> of A/CA/04/09 intranasally and swabs collected as indicated. Bars indicate frequency of influenza positive swabs by tissue culture (MDCK) virus isolation. Virus was shed primarily from nasal, oral, and tonsillar swabs up to 6 days after infection.



Swine were inoculated with 10e6 TCID<sub>50</sub> of A/CA/04/09 intranasally. Three animals were humanely euthanized on indicated days post-challenge and tissues collected for virus isolation as indicated. Bars indicate frequency of influenza positive tissues.

Future studies for the pandH1N1 focus on three areas. 1) Assessment of efficacy of human vaccines against pandH1N1 using the swine model of infection. 2) Assessment of efficacy of novel live-attenuated swine vaccines in pigs as a means to block reassortment and potential transmission to humans at the source species. 3) Assessment of novel, “universal” influenza vaccines against pandH1N1 and other influenza viruses. 4) Determination of the potential for reassortment of avian, human, pandH1N1, and swine influenza viruses in the pig, with specific endpoints including transmission, enhanced pathogenicity, and transfer of drug resistant and other virulence factors.

Future work on PRRSV is focused in one major area. We are currently collaborating with Dr. Biao He on development of novel vaccine vectors. One appealing construct is human parainfluenza virus 5 (PIV5, previously known as simian virus 5, SV5). We have previously shown that this vector can be engineered to express surface glycoproteins from other viruses, such as the hemagglutinin (HA) influenza virus. Insertion of the HA gene at different location within the genome regulates expression level. Interestingly, the HA is not only expressed in infected cells, but is also incorporated into the virus particle (see Tompkins *et al.* 2007 *Virology* 362(1):139-50). Infection with PIV5 causes no clinical disease and limited histopathology and incorporation of either an H3 HA (Tompkins *et al.*) or H5HA (data not shown) induces protective antibody and T cell responses. Recently, we have tested infectivity of PIV5 in swine. Similar to all other species tested, the virus infects pigs without causing disease (data not shown). For PRRSV, we propose to incorporate ORFs 3-6 individually or in combination to test the induction of cellular and humoral immune responses. Importantly, genetic features of PIV5 that modulate host immune responses have been identified. As an additional strategy, we plan to engineer PIV5 constructs with deletions in immunosuppressive viral genes to enhance immunogenicity of the vaccine if necessary.

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

none

**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.

See planned work above.

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

The major impacts for UGA efforts in this reporting period are only indirectly related to PRRSV. The two major impacts are the development of swine influenza and swine animals models in the

UGA investigators' labs and the recruitment of Dr. Biao He to UGA as a collaborating investigator to develop novel PRRSV vaccines. Both of these milestones should translate to significant progress in the next reporting period.

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

##### **1) Refereed publications**

none

##### **2) Abstracts or Proceedings**

none

##### **3) Book chapters or monographs**

none

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

##### **1) Current none**

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

As noted above, future work on PRRSV is focused in one major area. We are currently collaborating with Dr. Biao He on development of novel vaccine vectors. One appealing construct is human parainfluenza virus 5 (PIV5, previously known as simian virus 5, SV5). We have previously shown that this vector can be engineered to express surface glycoproteins from other viruses, such as the hemagglutinin (HA) influenza virus. Insertion of the HA gene at different location within the genome regulates expression level. Interestingly, the HA is not only expressed in infected cells, but is also incorporated into the virus particle (see Tompkins *et al.* 2007 *Virology* 362(1):139-50). Infection with PIV5 causes no clinical disease and limited histopathology and incorporation of either an H3 HA (Tompkins *et al.*) or H5HA (data not shown) induces protective antibody and T cell responses. Recently, we have tested infectivity of PIV5 in swine. Similar to all other species tested, the virus infects pigs without causing disease (data not shown). For PRRSV, we propose to incorporate ORFs 3-6 individually or in combination to test the induction of cellular and humoral immune responses. Importantly, genetic features of PIV5 that modulate host immune responses have been identified. As an additional strategy, we plan to engineer PIV5 constructs with deletions in immunosuppressive viral genes to enhance immunogenicity of the vaccine if necessary. Preliminary studies are also underway with alternate vaccine vectors.