

Project 2: Four Pig Pathogenesis Study with the 2009 A/H1N1 Influenza A Virus

Purpose of study: An important concern is to address whether meat, blood and tissue from pigs infected with the new 2009 H1N1 Influenza A Virus is free of infectious virus.

Experiment:

- Four 5-week-old cross-bred pigs from a herd free of swine influenza virus (SIV) and porcine reproductive and respiratory syndrome virus (PRRSV) were housed in containment facilities and cared for in compliance with the Institutional Animal Care and Use Committee of the National Animal Disease Center (NADC).
- Pigs were inoculated intra-tracheally with an infective dose of the 2009 H1N1 Influenza A Virus isolated from persons in California (A/CA/04/2009) obtained from the Centers for Disease Control and Prevention (CDC).
- Pigs were observed daily for clinical signs of disease. Nasal swabs were taken on 0, 1, 2, 3, 4, and 5 days post infection (dpi) to evaluate nasal shedding. Pigs were humanely euthanized on 5 dpi, which is considered the peak of infection in the NADC porcine SIV challenge model, to evaluate lung lesions and viral load in the lung and tissues. Fresh samples were taken from lung, tonsil, inguinal lymph node, liver, spleen, kidney, skeletal muscle (ham), and colon contents (feces), and examined using both real time RT-PCR and virus isolation (VI) techniques, which are the most sensitive and specific tools to detect the presence of viral nucleic acid and live virus, respectively.

Results:

- Tissues outside the respiratory tract were found to be negative by VI at 5 days post infection. Only respiratory tract samples were positive by both methods (real time RT-PCR and VI).
- The inguinal lymph node from one pig and serum from two pigs were positive by real time RTPCR. However, lymph node and serum samples from all pigs were negative by VI.
- By contrast, all day 5 post infection nasal swabs and lung lavage fluids were positive by real time RT-PCR and VI, and lung tissue homogenates from all four pigs were positive by real time RT-PCR and 2/4 samples positive by VI.

Conclusion: Live 2009 A/H1N1 Influenza A Virus was only detected in the respiratory tract of infected pigs and the virus does not appear to spread and replicate in other tissues based on the day 5 post infection samples.

Next Step: A larger study will be conducted to evaluate tissues by VI and real time RT-PCR that will include additional time points (1, 3, 5, and 7 days dpi).