RESEARCH REPORT
SWINE HEALTH

Title: Contribution of prior SIV infection in enhancing secondary *Haemophilus parasuis* disease - NPB #09-127

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Date Submitted: July 25th, 2011

Industry Summary:
Porcine respiratory disease complex (PRDC) is a multifactorial disease process with a variety of infectious agents contributing to disease. The complex cannot be simply explained as infection with multiple pathogens as other factors, such as immune status and management practices contribute to disease occurrence and severity. Swine influenza virus (SIV) is a known contributor to PRDC and may predispose to secondary bacterial infection. The time following SIV infection in which a pig remains susceptible to secondary bacterial disease is unknown, and is an important point from a management perspective. To determine if SIV predisposes or enhances secondary *Haemophilus parasuis* (Hps) infection, studies were performed to evaluate disease severity to Hps challenge in pigs previously infected with SIV. Two separate studies were performed in which pigs were challenged with Hps 5 or 10 days following SIV infection. In the first study, 4-week old pigs were challenged with SIV and 10 days later, infected with Hps. There was no significant difference in Hps colonization in SIV/Hps-infected or Hps-only infected pigs, although host immune responses were significantly increased in the SIV/Hps group compared to the Hps- or SIV- alone groups. In the second study, in an attempt to bypass maternal immunity, 8-week old pigs were used. Pigs were challenged with SIV and then 5 days later, challenged with Hps. Hps colonization 1 day following Hps challenge was not significantly effected by prior SIV infection, nor were host immune responses significantly different between SIV/Hps challenged pigs compared to Hps-only. However, the Hps challenge was virulent, as disease in both the SIV/Hps group and Hps-only group was severe enough to warrant euthanasia of the pigs prior to the scheduled necropsy. Lesions were consistent with Glassers disease, and Hps was recovered from several systemic sites. The results from these studies highlight the need for methods to evaluate Hps immune status, both for research studies as well as field susceptibility. Results regarding the susceptibility to secondary bacterial infection following SIV are mixed, but our results did generate a model that has now been used to evaluate Hps pathogenesis and associated virulence factors.

Scientific Abstract:
Porcine respiratory disease complex (PRDC) is a multifactorial disease process that causes significant loss in the swine industry. Factors contributing to the disease process include a variety of infectious agents, such as swine influenza virus (SIV) and *Haemophilus parasuis* (Hps), and factors such as herd immune status and management practices. The disease process is not simply explained by infection with multiple pathogens, and the contribution of each infectious agent and timing of infection likely contribute to the disease process, but are difficult to model. Influenza virus has been shown to predispose other animal species to secondary bacterial infection; thus, studies were completed in pigs to evaluate the contribution of SIV infection to secondary Hps disease. Two separate studies were performed in which pigs were first inoculated with SIV and secondarily challenged with Hps, either 5 or 10 days following SIV challenge (8-week old versus 4-week old, respectively). In 4-week old pigs, Hps colonization was not altered by prior SIV infection (10-days before Hps), although cytokine responses by tracheal epithelial cells were increased in the SIV/Hps challenged pigs. We were unable to evaluate responses in 8-week old pigs challenged with Hps 5 days following SIV because the strain of Hps used for these studies was virulent in 8-week old pigs. These data indicate that host immune status plays a significant role in disease susceptibility. While not surprising, it does highlight the need for methods to evaluate host immunity to Hps, especially when designing studies with Hps challenge. In conclusion, co-infection can lead increased host immune responses at the local level and studies are ongoing to understand the mechanism by which this occurs.
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Introduction:
It is well known that swine influenza virus (SIV) infection alone causes significant disease characterized by respiratory distress and poor growth. However, it is also well appreciated that SIV plays a significant role in the porcine respiratory disease complex (PRDC), though the mechanism in which this occurs is not clearly defined. *Haemophilus parasuis* (Hps) infection is often mild if clinical disease even occurs. It has been shown that pigs often carry Hps in the nose without exhibiting any disease. However, infection with some strains of Hps can result in pneumonia, whereas infection with other strains results in systemic Glasser's disease. The mechanism in which different strains can cause different disease states is currently unknown. Vaccination to individual pathogens contributing to PRDC is one mechanism of control the condition, however, vaccination strategies to control both SIV and Hps are difficult due to the large number of circulating strains and poor cross-protection between strains. Thus, housing and management practices will play an important role in controlling PRDC resulting from SIV infection. The experiments performed for this proposal were designed to determine how long following SIV pigs remain susceptible to secondary Hps disease. The information will provide information to the swine industry for management practices following an SIV outbreak. For example, if pigs that have recently suffered from SIV (even if the virus has been cleared), are introduced with animals in which it did not have prior exposure, the recovering pig may contract Hps colonizing the nasal passages of the new pigs in which it has no prior immunity. The recent SIV infection in which it is recovering from is likely to make the pig susceptible to secondary

bacterial pneumonia. Thus, to develop effective management practices, it is important to know how long following SIV infection pigs continue to exhibit enhanced susceptibility. It has been shown for other animal species that SIV can alter secondary host responses, which likely plays a role in this phenomenon.

Objectives

Objective 1 - During the SIV recovery period, is pulmonary disease exacerbated and host immune responses significantly altered following secondary Hps challenge?
Objective 2 - Does recent, yet cleared, SIV infection enhance secondary Hps disease?

Materials and Methods

Two separate studies were performed for fulfilling the objectives and objective 2 experiments were performed before the objective 1 experiment. In the first study, sows were purchased from farm A and delivered to the NADC for farrowing on site. Piglets were weaned at approximately 7 days of age and distributed into 4 groups of 14, blocking by litter. At 4-weeks of age, 28 piglets were challenged with SIV by the intranasal route. Ten-days following SIV inoculation, 14 of the SIV-inoculated piglets and 14 naïve piglets were challenged with Hps strain 12939 by the intranasal route. One day following Hps inoculation, 7 pigs from each of 4 treatment groups (non-infected, SIV-only, Hps-only, SIV/Hps) were necropsied to evaluate lung lesions, Hps burdens and host immune responses. Hps in the nose, trachea, and lung were enumerated and cytokine mRNA levels in alveolar macrophages and trachea epithelial cells were evaluated.
In the second study, sows were purchased from farm B and farm C (couldn't fill complete order from single farm) and delivered to the NADC for farrowing on site. Piglets were weaned at approximately 7 days of age and distributed into 4 groups of 16, blocking by litter. At 8-weeks of age, 32 pigs were challenged with SIV by the intranasal route. Five-days following SIV inoculation, 16 of the SIV-inoculated pigs and 16 naïve pigs were challenged with Hps strain 12939 by the intranasal route. One day following Hps incoluation, 8 pigs from each of 4 treatment groups (non-infected, SIV-only, Hps-only, SIV/Hps) were necropsied to evaluate lung lesions, Hps burdens and host immune responses. Hps in the nose, trachea and lung were enumerated and cytokine mRNA levels in tracheal epithelial cells and lung lavage cells were evaluated

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Results

Objective 2 - Hps colonization in the trachea was significantly increased in pigs challenged with SIV 10 days prior to Hps infection within a day of Hps challenge. However, by day 7 following Hps challenge, there was no significant difference in Hps colonization in the nose, trachea or lung regardless of prior SIV challenge. Lung lesions were not different between treatment groups at either necropsy date. mRNA levels of IL-6, IL-8 and MCP-1 were significantly increased in the tracheal epithelial cells of SIV/Hps challenged pigs. There were no significant differences in cytokine mRNA levels of lung lavage cells between treatment groups, though all were significantly increased over cells from non-infected pigs.
Objective 1 – The results from this objective are significantly confounded by the Hps challenge. Pigs were not challenged with SIV until 8 weeks of age, and 5 days later, challenged with Hps. A group of pigs challenged with Hps-only was also included. On day 1 following Hps challenge, there was no significant difference in Hps numbers in the nose, trachea or lung, regardless of prior SIV infection. There was no significant difference in mRNA levels of IL1β, TNF-α or IL-8 in lung lavage cells though levels were significantly increased in pigs challenged with Hps

(regardless of SIV) when compared to mRNA levels in cells collected from pigs challenged with SIV-only. These results were consistent with protein levels of the same cytokines in the lung lavage.

By day 2 following Hps challenge, the pigs scheduled for necropsy on day 7 began to show severe clinical signs of disease if they were inoculated with Hps, regardless of prior SIV inoculation. Clinical signs included depression, reluctance to rise, and labored breathing. In accordance with animal care regulations, pigs were euthanized prior to the scheduled necropsy. Upon post-mortem examination, signs of Glasser's disease were apparent (pleuritis, pericarditis, peritonitis, cloudy meninges) and Hps was recovered from several systemic sites. Six of 8 in both the SIV/Hps group and Hps-only group died prior to scheduled day 7 necropsy.

Discussion:

Work completed in this proposal highlights the difficulties in studying viral/bacterial co-infection interactions and the lack of testing available for determining *H. parasuis* carriage and immunity. Current serology for *H. parasuis* is only successful at detecting antibody if the pigs survived a systemic infection. Maternal antibody may contribute to protection early in life and may have provided some level of protection to the pigs challenged at 4 weeks of age. However, it's likely that maternal antibody waned by 8 weeks of age and pigs in that experiment were then susceptible to the Hps challenge. The different sources of sows for piglets may have also contributed to susceptibility.

Results from this work may not have completely addressed the proposed objectives due to experimental complications, but did further our understanding of *H. parasuis* disease and provide information for a conventional model to study *H. parasuis* disease. To date, *H. parasuis* has typically been studied in cesarean-derived, colostrum-deprived (CD/CD) pigs, which requires surgical facilities and intense rearing of the piglets. The model developed from work in this grant, in which pigs are weaned early, fed-milk replacer, and challenged with *H. parasuis* after 8 weeks of age, has now been used for additional *H. parasuis* studies. It is worth noting that the sows must come from a specific herd for this model to work and we are trying to understand why that is (carriage for example). In addition, our research group is studying Hps pathogenesis and virulence by sequencing a number of Hps strains and this work has led to a model for evaluating virulence *in vivo*.