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Association of the presence of influenza A virus and porcine reproductive and respiratory syndrome virus in sow farms with post-weaning mortality

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ABSTRACT

Influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV) are among the most important pathogens affecting pigs worldwide. Their effect on post-weaning mortality can be substantial and may be potentiated by other concomitant factors. Here, the objective was to evaluate the association between IAV and PRRSV infection at weaning with post-weaning mortality observed in wean-to-finish farms in order to better quantify the full impact of their presence in breeding herds.

IAV and PRRSV presence was assessed by real time reverse transcription (RRT)–PCR on oral fluid samples from suckling piglets in nine sow farms. Production data from 177 batches of growing pigs weaned one week before/after IAV and PRRSV testing were analyzed to measure the association between IAV and/or PRRSV test results and mortality recorded for a given batch through the use of Bayesian mixed effects negative binomial multivariable regression model. The model accounted for potential confounders such as flow, date at weaning, days on feed and batch size. A statistically important association between IAV (incidence ratio (IR) = 1.18, 95% posterior probability interval 1.15–1.21) and PRRSV (IR = 1.41, 95% PPI 1.30–1.52) with post-weaning mortality was detected, with season and number of days on feed also associated. Our results suggest that infection with IAV or PRRSV in the pre-weaning period is associated with an increase in post-weaning mortality. This association should be taken into consideration when measuring the impact of IAV and PRRSV in breeding herds.

1. Introduction

Influenza A virus (IAV) is one of the most common respiratory pathogens affecting swine in large parts of the world (Olsen et al., 2006). IAV is endemic in pigs in North America where infection occurs throughout the year (Corzo et al., 2013; Schultz-Cherry et al., 2013). The virus typically spreads rapidly through a susceptible population due to its high transmissibility and the short incubation period that precedes shedding. Infection results in clinical signs of fever, anorexia, prostration, sneezing, coughing, and weight loss, that range from mild to severe (Olsen et al., 2006). Even though mortality directly attributed to IAV infection across ages is considered to be low (<1%), other factors such as co-infections and impaired immune status, may increase the severity of the disease (Olsen et al., 2006).

Control of IAV infection can be attempted by vaccination, but the variety of virus lineages and strains is a challenge that can jeopardize the effectiveness of this strategy (Van Reeth and Ma, 2013). IAV transmission is not fully understood, but virus circulation within breeding herds may be intermittent and reflected in positive test results followed by negative results (Panyasing et al., 2014). Transmission dynamics are easier to study in wean-to-finish (WF) sites subjected to all-in all-out practices where piglets may arrive already infected but new susceptible individuals are not regularly added to the population, and therefore population is more homogeneous (Reynolds et al., 2014). Given the difficulties to eliminate IAV from breeding herds, infection of suckling piglets in

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the presence of maternally derived antibodies may reduce disease impact compared to infection later in the growing phase. However, re-infection may still occur and, in fact, maternal immunity may preclude the development of an active immune response to the infection thus leading to susceptibility to future reinfections (Loeffen et al., 2003). Therefore, it is important to evaluate the effect of IAV infection at an early age on performance of the growing pigs. Indeed, co-infection with more than one viral or bacterial agent, such as porcine reproductive and respiratory syndrome virus (PRRSV), is not uncommon in cases of porcine respiratory disease (Choi et al., 2003).

Despite its relatively short history, PRRSV is also among the most important pathogens affecting the swine industry around the world with an estimated annual cost of $664 million dollars in the US alone (Holtkamp et al., 2013). Even though the main impact of PRRSV infection is due to the occurrence of reproductive failure and increased preweaning mortality (Zimmerman et al., 2006), PRRSV has been also associated with respiratory issues and increased post-weaning mortality (Dewey et al., 2006), with *in-vivo* and *in-vitro* data suggesting that co-infection with IAV may increase the severity of disease (Van Reeth et al., 1996; Kitikoon et al., 2009; Dobrescu et al., 2014). Control and elimination of PRRSV in breeding herds can be pursued using different strategies with the common goal to wean non-infected piglets (Corzo et al., 2010). In fact, absence of PRRSV in piglets at weaning has been proposed as an indicator of the progress achieved towards control or elimination of the virus (Holtkamp et al., 2011).

In the study here, we quantified the association of IAV and/or PRRSV infection in suckling pigs in breeding herds with mortality observed in the growing phase. Post-weaning mortality was chosen as the outcome of interest because it is easily quantified and reflects the economic cost associated with disease (Serrano et al., 2014). A Bayesian approach was chosen to evaluate the impact of using a censored prior for the effect of PRRSV on mortality. Results from this study will help to quantify the economic impact of, arguably, two of the most important diseases of commercial pigs in North America and worldwide.

2. Material and methods

2.1. Study population

A cohort of 9 sow farms in the Midwest region of the United States was selected based on the willingness of farm owners to participate in the study and share their data. All sow farms were managed by one firm and were relatively similar to each other in terms of management practices. Performance records from all pig batches weaned into wean-to-finish (WF) sites between June 2011 and April 2014 were included. Pigs that were weaned directly into a nursery site first, and then moved into a finishing site were removed from the analysis to control for biases associated with management conditions specific of those sites. In addition, only those batches of pigs weaned within one week before or after collecting samples for IAV diagnostic testing (up to seven days apart from the sample collection) were included in the final dataset. Because weaned pigs from two different sow farms were commingled in the same barn at weaning, we use the term "flow" instead of "breeding herd", and refers to the origin of a given pig batch (that may include one or more sow farms).

2.2. Diagnostic techniques

Detection of IAV: oral fluid samples collected from four different ropes located in four farrowing pens from breeding herds were tested monthly for IAV detection, so that each oral fluid sample represented a farrowing pen. Only piglets had access to the ropes. Samples were subjected to RNA extraction using a commercial kit (MagMAX^{TM}-96 Viral RNA Isolation Kit, Life Technologies Corp., Carlsbad, CA), and presence of IAV was evaluated using real time reverse transcription polymerase chain reaction (RRT–PCR) directed at the highly conserved matrix gene (Slomka et al., 2010).

Detection of PRRSV: the same RNA extractions obtained from oral fluid samples used for IAV detection were also tested for PRRSV using a commercially available single-tube RRT–PCR assay (EZ-PRRSV MPX 4.0, Tetracore Inc., Rockville, MD). PRRSV breeding herd classification followed the guidelines published by Holtkamp et al. (2011) with modifications. Briefly, sow farms were classified as positive if oral fluids tested RRT–PCR positive; stable if oral fluids collected over a 90-day period tested RRT–PCR negative; and negative if results from oral fluids collected over a 90-day period tested negative and seronegative gilts introduced to the farm remained seronegative for at least 60 days (Holtkamp et al., 2011)

The unit of analysis here, the batch, was considered positive to either IAV or PRRSV if at least one sample (i.e., one rope) tested positive in the RRT–PCR analysis.

2.3. Data analysis

The following information from the batches fulfilling the inclusion criteria were included in the final dataset: IAV testing results (negative/positive), PRRS breeding herd status classification when that batch was weaned [negative (including both negative and stable status) or positive], beginning and end dates of the WF period, pig source (flow), number of pigs in a the batch, and mortality (percentage of dead pigs). Dates from start to finish of the WF period were used to: (a) calculate the total number of days on feed; and (b) to compute a dichotomous variable, referred to as "season". This was done to differentiate cold (September–February) *vs.* warm (March–August) temperature months since colder months have been associated with increased risk of respiratory disease (Brown, 2000). Distribution of mortalities in the selected batches was explored visually. Outliers were detected using the generalized extreme studentized deviate (ESD) test (Rosner, 1983) and removed from the database. Bivariate association between raw mortality (dependent variable) and IAV and PRRSV results, season, days on feed, batch size, and pig source (independent variables) were explored using Student t-test, ANOVA, and Pearson correlation coefficient. A Bayesian generalized linear mixed effects regression model (Breslow and Clayton, 1993) was fitted to evaluate the association between the number of deaths in a given batch and the independent variables. Continuous variables (days on feed and batch size) were alternatively entered into the models untransformed (raw data), log-transformed, and categorized into quartiles. The random variable $O_{i,j}$ representing the number of deaths recorded for each batch *j* from flow *i* was assumed to follow a Poisson distribution, with a mean $\mu_{i,j}$ representing the mean number of deaths of batch *j* from flow *i*. This mean is the product of the expected number of deaths $E_{i,j}$, calculated as the overall mortality across all batches times the number of animals in batch *I*, times the batch-specific relative risk of death, $\varphi_{i,j}$, such that

$$O_{i,j} \sim P\left(\mu_{i,j}\right) \quad (1)$$

$$\log\mu_{i,j} = \log E_{i,j} + \log\varphi_{i,j}$$

with

$$\log\varphi_{i,j} = \alpha_i + \beta_1 X_{ij1} + \beta_2 X_{2ij} + \ldots \beta_k X_{ijk}$$

where X_{ijk} denotes the k^{th} covariate with the corresponding regression parameter β_k and α_i represents the random effect (intercept) corresponding to the *i* flow batch. Alternatively, a negative binomial

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