ONTARIO PORK

Ontario Pork Research Final Report (17-011) Executive Summary

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Introduction: Control of PRRSV and associated co-infections is often difficult due to the wide assortment of bacterial and viral pathogens affecting commercial farms, and vaccines that are less than 100% effective. Alternatives strategies to reduce the impact of disease on performance are needed. One such strategy involves the selection of breeding stock with greater resilience to diseases. To enable this, biomarkers of disease resilience are needed that preferably can be measured in high health nucleus farms but correlate with disease resilience in commercial farms. This research, funded jointly by Ontario Pork and NSERC, and utilizing valued resources provided PigGen Canada, the PRRSV Host Genetics Consortium (PHGC) and Pregnant Gilt Model, investigated two potential markers of resilience.

Objectives: Our overarching objective was to evaluate sCD163 and thyroid hormones, T3 and T4, as potential serum biomarkers used to select genetically superior animals with increased resilience to PRRSV and other related co-infections.

Materials and Methods: Levels of sCD163, T3 and T4 were measured in sera from four sera datasets obtained from: a) healthy animals from three Ontario farms, b) pregnant gilts following PRRSV challenge, c) nursery pigs following PRRSV challenge, d) nursery-finisher pigs following polymicrobial challenge. Genome wide association studies were conducted.

Results and Discussion: sCD163 levels are stable in healthy gestating females but steadily decrease following weaning in healthy NGF pigs. In PRRSV challenges pregnant gilts, levels are lower in susceptible versus resilient animals. High pre-challenge (resting) levels were associated with decreased ADG following experimental PRRSV challenge. Greater sCD163 shedding response was associated with lower ADG following polymicrobial challenge. Both T3 and T4 levels in serum were suppressed following PRRSV and polymicrobial natural challenge. Thyroid hormone suppression was associated with decreased ADG in NGF pigs, and with high viral load and meconium staining in fetuses following PRRSV challenge. Two completed genome wide association studies (GWAS) identified several SNPs related to thyroid hormone levels.

Conclusions: Our results provide evidence to conclude that sCD163 and the thyroid hormones measured in serum may be biomarkers of disease resilience in breeding and/or feeding pigs.

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Introduction:

Porcine reproductive and respiratory syndrome virus (PRRSV) and PRRSV-induced co-infections continue to cause substantial economic losses in the swine industry. Although considerable variability in the host response to PRRSV exists, there are currently no reliable phenotypes or serum biomarkers for predicting disease severity on which to base selection of commercial pigs for disease resilience. To combat this shortcoming, we collaborated with PigGen Canada (PGC) and the Porcine Host Genetics Consortium (PHGC) to investigating potential serum biomarkers (soluble CD163 and thyroid hormones) in both breeding animals and growing pigs. With funding was provided by Ontario Pork and NSERC (Collaborative Research and Development Grant), this research exploited an extensive repository of existing sera and new samples collected from three Ontario farms and from a Natural Disease Challenge (NDC) trial underway at the CDPQ in Deschambault, Quebec.

CD163 is a scavenger protein involved in the elimination of highly toxic haemogoblin during the natural turnover of red blood cells[1]. It also serves as the obligate receptor for PRRSV and is necessary for viral entry into the cell[2, 3]. CD163 has a soluble portion outside of the cell, soluble CD163 (sCD163), which when cleaved from the cell moves freely into tissues and blood[4]. The presence of sCD163 in humans is associated many inflammatory disorders, chronic diseases, and bacterial and viral infections[5]. Diseases of swine, such as African swine fever (ASF), have been shown to induce sCD163 shedding upon infection[6].

Thyroid hormones are vital for regulating appetite[7], metabolism and growth[8]. During pregnancy, these hormones play a critical role promoting fetal survival and development[9]. We have recently demonstrated that levels of the thyroid hormones (T3 and T4) are depressed following PRRSV infection in all ages of pigs[10, 11] and the severity of suppression related to the reduction in growth rate in growing pigs and potentially viability in late gestation fetuses.

Objectives:
Our overarching objective was to evaluate sCD163 and thyroid hormones as potential serum biomarkers that could be used to select genetically superior animals with increased resilience to PRRSV and other related co-infections. This research had two phases that interconnected through common sample and phenotypic data sets, industry partners, and research teams. Sponsored by Ontario Pork and NSERC, phase 1 assessed the utility of sCD163 whereas Phase 2 assessed the utility of thyroid hormone levels. In each phase, multiple sera sets were used to specifically investigate resting (pre-infection) and response (post-infection) levels of these two biomarkers in healthy pigs from commercial farms, and in pregnant gilts, fetuses and nursery/finisher pigs following PRRSV or natural polymicrobial challenge.

Materials and Methods:
Phase 1: sCD163 levels were measured by an in-house developed ELISA[12] in four sets of archived sera: a) ~90 healthy gestating females (blocked by parity and gestation age) and ~90 healthy wean-finish pigs (blocked by age) from each of 3 Ontario farms; b) 60 susceptible or resilient pregnant gilts following PRRSV challenge in 3rd trimester; c) 150 susceptible or resilient nursery pigs post PRRSV infection from six PHGC experiments using two viral strains; and d) 225 nursery-finisher pigs from 3 batches supplied by three Ontario farms (as in 1a) following natural polymicrobial challenge including multiple strains of PRRSV, *Mycoplasma hyopneumoniae*, and other common viral and bacterial pathogens.
Phase 2: Thyroxine (T4) and/or triiodothyronine (T3) were measured by radioimmunoassay (RIA) using commercial kits (MP Biologicals, CA) in the same four sets of archived sera: a) ~80 healthy gestating females (blocked by parity and gestation age) and 50-60 healthy wean-finish pigs (blocked by age) from each of 3 Ontario farms; b) 1358 fetuses at 12 (n=491) or 21 (n=861) days post maternal PRRSV challenge in 3rd trimester; c) 190 susceptible or resilient nursery pigs post PRRSV infection from PHGC experiments; and d) ~200 nursery-finisher pigs following natural polymicrobial challenge. **Genome Wide Association studies (GWAS)**: *A) Respiratory PRRS*: While we proposed testing an additional ~900 pigs from the polymicrobial trial, we elected to use the PHGC trials instead because the challenge was better defined and consistent across all pigs. Moreover, rather than testing two time points in 900 pigs, we tested one timepoint in 1800 pigs allowing inclusion of data from both viral strains. Therefore, T3 levels at 11 DPI were quantified in 1862 PHGC pigs in total. Genotyping was performed using the Porcine 60K chip. Two statistical models were evaluated including fixed effects: sex, WUR SNP, viral load and trial number or virus strain. *B) Reproductive PRRS*: Because the ~1350 fetuses were genotyped using two different SNP chips (Porcine 60K or the Affymetrix Pig HD 600K), it was necessary to identify and filter the data that were common to both chips before proceeding with the analyses. The

statistical models (T3 and T4) included as fixed effects: viral load in thymus, sex, DPI and inoculation status. This resulted in 46,370 SNPs across 1,276 fetuses being used for downstream analyses.

Results and Discussion:

Phase 1: Soluble CD163

1a) Healthy pigs: In gestating females, sCD163 levels did not differ by parity or stage of gestation but the variance was greater in older sows (P3-5) (Fig 1A). In nursery-finisher pigs, sCD163 levels were greatest in pre-weaned pigs in serum steadily decreased over 12-16 weeks before stabilizing (Fig 1B). These data suggest that some older sows may have a greater propensity to shed CD163 or are more reactive to inflammatory insults. We hypothesize that inflammatory stimuli in the environment, stress and/or the introduction to dry feed may elevate sCD163 levels around the time of weaning.

1b) Pregnant gilts following PRRSV challenge: sCD163 levels were undetectable in sera in 25 of the 60 gilts at one or more time points; 11 between 0-6 dpi and 20 at 19 dpi. It is noteworthy that 18/25 (72%) of the undetectable gilts were in the susceptible category. Mean sCD163 levels were decreased in susceptible compared to resilient gilts (Fig 2), categorized based on percent fetal viability and viral load.

1c) Nursery pigs following PRRSV challenge: sCD163 decreased over 11 days following PRRSV infection (Fig 3A) similar to the trend observed in healthy pigs post weaning. We hypothesized that sCD163 levels would increase following PRRSV infection but no acute shedding response to the viral infection was seen. While there were no significant differences among the resilience groups (selected on the basis of weight gain and viral load following PRRSV challenge) pigs with low resting (D0) sCD163 levels showed greater shedding response following infection (Fig 3B) and greater weight gain (D0-21)(Fig 3C). These results suggest low resting sCD163 levels at weaning could be a biomarker of PRRSV resilience.

1d) Nursery-finish pigs following polymicrobial challenge: Soluble CD163 levels decreased during the isolation period (Fig 4A; D-14 to D0) similar to that noted in health pigs from commercial farms. Following polymicrobial challenge starting at D0, a shedding response was observed that peaked at 14 days post exposure (Fig 4A; D0-14). The magnitude of the shedding response during the first 42 days post challenge was negatively associated with ADG during the first 3 weeks post challenge (Fig 4B), but not beyond that.

Phase 2: Thyroid hormones (T3 and T4)

2a) Healthy pigs: Overall T3 and T4 levels differed by farm in both gestating and feeding pigs, so further analysis accounted for clustering by farm as a random effect. In gestating sows, levels of T3 and T4 were lower in late than early or mid-gestation (Fig 5) but did not differ by parity. In the feeding herd, neither T3 nor T4 levels differed across age with one exception; T4 levels were significantly lower in week 8 versus 20 (Fig 6).

2a) Fetuses following maternal PRRSV infection: Fetuses were categorized into susceptible and resilient groups based on the fetal preservation and thymic viral load (Fig 7). T3 and T4 levels in fetal serum were decreased in high viral load and meconium stained fetuses (Fig 8). The relationship was consistent in 12 and 21 dpi fetuses, but more pronounced at 21 dpi. Although we have previously reported thyroid hormone levels decrease in high-viral load fetuses[10] the substantial number of fetuses included in this present study confirmed a decrease in T3 and T4 in low viral load MEC fetuses as well suggesting fetal susceptibility may be independent of viral load.

2c) Nursery pigs following PRRSV challenge: Using Z-scores of 0.75 for weight gain (W) and viral load (V), between 25-70 pigs were identified in each of the four resilience categories: tolerant (HVHW), susceptible (HVLW), resilient (LVHW) and ultra-susceptible (LVLW)(Fig 9). Across all groups, T3 and T4 levels were decreased from 4 to 11 dpi (Fig 10). The levels of thyroid hormone were then assessed at peak disruption (7 and 11 dpi) using a model including resistance, resilience and viral strain. T3 and T4 decreased significantly in relation to total weight gain but not viral load (Fig 11).

2d) Nursery-finish pigs following polymicrobial challenge: Susceptible and resilient animals were identified based on within-batch Z-scores for ADG from entry to 61 days post challenge using a cutoff of 0.75 SD. Across all groups (low, mid, high ADG) levels of T3 and T4 were significantly depressed post challenge (Fig 12). T4 remained depressed at 42 days post challenge where as T3 levels showed evidence of a rebound by day 42. The degree of post entry depression in both thyroid hormones was positively associated with growth performance (Fig 13).

2e) GWAS – Respiratory PRRSV: After amalgamating available phenotypic and genotypic data and appropriate quality control, 1792 pigs from 12 PHGC batches (range 35-194 per batch) were included in the dataset. Seven batches had been challenged with NVSL and 5 batches with KS06 viral strains. From two statistical model, 5-6 SNPs on various chromosomes were identified that each explained >2% of the total genetic variance, with the most prominent accounting for >6%. Further investigation of the individual genes in close proximity to these SNPs is underway.

2e) GWAS – Reproductive: Missing genotypes have been imputation and the statistical model determined. Analysis to generate the genomic relationship matrix, calculate genomic parameters and the GWAS in GenSel is ongoing and results are expected within weeks.

Conclusions:

There is evidence to conclude that both sCD163 and the thyroid hormones are potential biomarkers of disease resilience. At present, we have stronger evidence for the thyroid hormones and supporting further investigation for potential use in breeding programs. That said, we do not want to rule out sCD163 at this time. While further investigation is needed, high resting (pre-challenge) levels of sCD163 appear to be associated with lower weight gain following PRRSV challenge (PHGC) and greater sCD163 shedding response appear associated with lower ADG following polymicrobial challenge. In pregnant gilts, the opposite trend was evident with susceptible gilts with higher fetal mortality and fetal viral load showing lower sCD163 levels at pre- and post- late pregnancy PRRSV challenge. The considerable variation in sCD163 levels in gestating sows, particularly in older sows, provides an opportunity to further investigate associations with productivity in healthy farms and in PRRSV outbreaks.

There is overwhelming evidence that both thyroid hormones, T3 and T4, are suppressed following PRRSV infection. This occurs in all age groups including pregnant gilts (and presumable sows as well although not evaluated), near term fetuses, and nursery pigs. Levels also decrease following natural polymicrobial challenge. The degree of suppression is associated with ADG in nursery-finisher pigs, with earlier rebound associated with improved ADG. Following PRRSV challenge, both thyroid hormones are depressed in high viral load and meconium stained fetuses suggesting they are contributors to fetal demise.

Knowledge Transfer:

A) Presentation, poster or abstract from a scientific or industry meeting (please provide a copy or the link):

- Pasternak, J. A., et al. Thyroid hormone disruption in feeder pigs following experimental and natural PRRSV2 and polymicrobial infections. Proc. North American PRRS Symposium. Chicago, IL. November 1-2, 2019. Pg 36 (Poster)
- Pasternak, J. A., et al. Maternal and fetal thyroid hormone disruption following late gestation PRRSV2 challenge. Proc. North American PRRS Symposium. Chicago, IL. November 1-2, 2019. Pg 37 (Oral)
- Pasternak, A., et al. Measurement of soluble CD163 in pig serum to assess host responses following PRRSV infection. Proc. North American PRRS Symposium. Chicago, IL. December 1-2, 2018. PRRS19 (Poster).
- Forsberg, N., et al. Establishing utility of soluble CD163 as a biomarker for predicting PRRS-induced disease severity. WCVM Undergraduate Student Research Poster Day. Saskatoon, Canada, September 5-6, 2018 (Forsberg)
- Harding, J. et al. Establishing value/utility of soluble CD163 as a biomarker for predicting PRRS-induced disease severity/resilience in swine herds. PigGen Canada Research Update. Banff Alberta, January 9, 2018.
- Harding, J. et al. Establishing value/utility of soluble CD163 as a biomarker for predicting PRRS-induced disease severity/resilience in swine herds. PigGen Canada Research Update. Banff Alberta, January 8, 2019.

- Pasternak J. A. et al. Thyroid hormone disruption following PRRSV infection in dams and fetuses. PigGen Canada Research Update. Banff Alberta, January 8, 2019.
- Pasternak J. A. et al. The fetal response to late gestation Infection with porcine reproductive and respiratory syndrome virus. One Reproductive Health Group. Saskatoon SK, January 22 2020

B) Peer-reviewed scientific papers

- Pasternak, J. A., MacPhee, D. J., Harding, J. C. S. 2020. Maternal and fetal thyroid dysfunction following porcine reproductive and respiratory syndrome virus 2 infection. Vet. Res. 51:47.doi.org/10.1186/s13567-020-00772-2
- Pasternak, J. A., MacPhee, D., Harding, J. 2019. Development and application of a porcine specific ELISA for the quantification of soluble CD163. Vet. Immun. Immunopath. 210:60-67. doi.org/10.1016/j.vetimm.2019.03.011

C) Popular Press Articles and communications:

- None yet

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12. Pasternak JA, MacPhee DJ, Harding JCS. Development and application of a porcine specific ELISA for the quantification of soluble CD163. *Vet. Immunol. Immunopathol.* 2019;210:60-67.