

# Antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and Mycoplasma hyopneumoniae from weaning to the end of the finisher stage in fourteen groups of pigs in Ontario

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## Research

**Keywords:** porcine reproductive and respiratory syndrome, swine influenza, mycoplasmal pneumonia, PRRSV, IAV, Mycoplasma hyopneumoniae, ELISA, antibody response, swine

**Posted Date:** February 10th, 2020

**DOI:** <https://doi.org/10.21203/rs.2.22996/v1>

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# Abstract

## Background

Porcine reproductive and respiratory syndrome, swine influenza, and mycoplasmal pneumonia are some of the most prevalent respiratory diseases affecting swine farm productivity in Canada. Monitoring for the prevalence of the infectious agents associated with these diseases on farm may help to improve herd-specific control strategies and to minimize the impact of disease on commercial swine farms. The objectives of this study were to investigate antibody responses to porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) from weaning to the end of the finisher stage on a subset of commercial swine farms in Ontario and to examine the effects of nursery diet on antibody responses.

## Results

Serology found 8, 61, and 31% of pigs at weaning, 1, 31, and 22% at the end of nursery, 8, 38, and 18% at the end of grower, and 11, 48, and 25% at the end of the finisher stage tested seropositive for PRRSV, IAV, and *M. hyopneumoniae*, respectively. Of the groups tested for PRRSV, IAV, and *M. hyopneumoniae*, 3, 14, and 5 groups had > 20% of pigs that tested seropositive at least once over the course of production (“high seropositivity”). In general, seropositivity was more likely to be lower at the end of nursery compared to the other production stages for all three pathogens, and more likely to be higher for PRRSV and IAV at weaning, end of grower, and end of finisher. Pigs that were seropositive for PRRSV were more likely to be seropositive for *M. hyopneumoniae* ( $p < 0.001$ ). Overall, pigs fed a low complexity diet during nursery were more likely to be seropositive for PRRSV ( $p < 0.001$ ) and IAV ( $p = 0.04$ ).

## Conclusions

This study provides information regarding changes in serum antibody in pigs across different stages of production and highlights periods of vulnerability. Additionally, these findings may encourage further research into the effects of nursery diet complexity on disease susceptibility and immune response.

# Introduction

Infectious diseases on swine farms can have detrimental effects on both producer profits and animal health and welfare. Porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) are three of the most significant disease-causing pathogens affecting swine-farm productivity (1). Despite efforts to eradicate these pathogens and their associated diseases, they continue to be widespread in the world swine population and result in huge economic losses for pork producers.

One difficult aspect of disease eradication on swine farms is the variation in immunity throughout the lifecycle of the animals. As young pigs are weaned from the dam, their levels of maternal antibodies decline, after which pigs begin to produce their own antibodies as their immune systems develop (2). The initial decrease in antibody levels with waning passive immunity increases disease susceptibility in young pigs, and as such, it is important to monitor for the presence of pathogens at multiple stages of production in order to identify herd-specific patterns of infection so as to better protect immunologically naïve pigs from infection and avoid the spread of disease on farm.

Further complicating the issue of infection on commercial swine farms is the potential presence of additional disease-causing pathogens, which may take advantage of the already fragile immune system of an infected individual to co-infect their host (3). For example, *M. hyopneumoniae* adheres to cilia of the respiratory epithelium preventing clearance of cellular debris, thereby increasing the potential for co-infection (4–6). Similarly, PRRSV suppresses the innate immune response mainly through its selective tropism and subsequent destruction of pulmonary alveolar macrophages (PAMs) (7), which leads to the suppression or alteration of type I interferon (IFN) pathways (8), which may also increase the susceptibility for co-infection.

Not only does infection with certain pathogens increase the potential for further infection, but co-infection is often more severe than infection by either pathogen alone. Previous research has determined that *M. hyopneumoniae* infection increased and prolonged PRRSV-induced pneumonia(9), and pigs co-infected with both *M. hyopneumoniae* and IAV have been shown to exhibit more severe clinical disease than pigs infected singly with either agent (10), These interactions may further exacerbate declines in producer profits and animal welfare, and thus avoiding infection with multiple pathogenic agents on farm is crucial in order to minimize the impact of disease.

Another aspect of swine production that may influence animal health and producer profits is feed. Not only is feed the costliest aspect associated with pork production, but nursery diet costs are especially high due to the need for a highly palatable feed that will allow the immature gut of the pig to adapt from an easily digestible milk diet to solid, grain-based feed. Common industry practice recommends producers feed a series of starter feeds in order to slowly transition weanlings from expensive, complex diets containing milk products, fishmeal, etc., to less expensive diets consisting of simpler plant-based ingredients (11). This allows pigs to develop the necessary enzymes required for digesting the constituents found in adult diets (11,12). Additionally, the composition of nursery diets, particularly protein content and quality, may affect animal health, carcass quality, and growth rate (12). Nursery diets that substitute animal proteins with plant-based proteins may decrease costs associated with pork production without sacrificing meat quality or body weight (13) but the effects these diets may have on antibody responses, if any, are not yet fully understood (14).

Infections with PRRSV, IAV, and *M. hyopneumoniae* are frequently chronic and/or subclinical, and due to the many factors affecting the incidence of the associated diseases, monitoring for the presence and distribution of microorganisms on-farm is an important step in identifying herd-specific patterns of illness and susceptible populations in order to implement effective control programs. The use of serological assays such as enzyme-linked immunosorbent assay (ELISA) tests are frequently used to monitor antibody responses to infectious agents rather than testing for the presence of the pathogen itself (15), because they tend to be less expensive and are fast and efficient to perform on large numbers of samples, allowing for a broader picture of pathogenic threats to be painted on a farm-by-farm basis and providing producers with the means to administer tailored preventative medicines and treatments specific to their hazards.

The objectives of this study were: 1) to measure antibody responses to PRRSV, IAV, and *M. hyopneumoniae* in pigs from weaning until the end of the finisher stage; 2) to examine the relationship in antibody responses among those three pathogens; and 3) to determine the impact of a nursery diet that uses mostly plant protein compared to the typical complex animal protein-based diet on seropositivity to PRRSV, IAV, and *M. hyopneumoniae*.

## **Materials And Methods**

### **Study design**

The farrowing source and pig selection for this study have been previously described (14,16). Briefly, fourteen groups of 50–60 pigs originating from eight farrowing sources in Southwestern Ontario were selected. Two cohorts (Cohort 1 and 2) were included in the study from six of the eight farrowing sources, while the other two included only one cohort (Cohort 1). Pigs in Cohort 1 were born between May and August, while pigs in Cohort 2 were born between October and January. All sources but one utilized off-site nursery and finishing, while the other was farrow-to-finish for Cohort 1 and off-site finisher for Cohort 2. Pigs received either a standard high-complexity (HC) nursery diet or experimental lower-cost, low-complexity (LC) diet in which the majority of the animal protein was replaced with plant protein throughout the nursery phase (13). The diets for all pigs were the same at all other production stages. Surveys were provided to producers to obtain information regarding farm management practices.

### **Sample collection**

Blood samples were collected from pigs at weaning and at the end of the nursery, grower, and finisher stages in all 14 groups except for one, where samples were not collected at the end of the finisher stage. Blood samples were collected from either the

jugular vein or suborbital sinus and centrifuged at 1500 x g for 20 minutes. The serum samples were then stored at -20 °C.

## Enzyme-linked immunosorbent assay (ELISA)

Sera were analyzed for the presence of PRRSV, IAV, and M. hyopneumoniae antibodies using three commercially available ELISA Kits (IDEXX Laboratories, Inc., Westbrook, Maine, USA) as per the manufacturer's instructions. Groups were classified as high or low seropositivity for each pathogen if at least 20% of pigs in a group were seropositive for that pathogen at least once over the course of production.

A sample-to-positive (S/P) ratio for PRRSV antibodies was calculated as follows:

$$S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}}$$

A pig was considered seropositive for PRRSV if the S/P ratio was  $\geq 0.4$ .

A sample-to-negative (S/N) ratio for IAV was calculated as follows:

$$S/N = \frac{\text{Sample absorbance (650)}}{\text{Mean}_{\text{negative control}}}$$

A pig was considered seropositive for IAV if the S/N ratio was  $< 0.6$ .

A S/P ratio for M. hyopneumoniae was calculated as follows:

$$S/P = \frac{\text{Sample absorbance (650)} - \text{Mean}_{\text{negative control}}}{\text{Mean}_{\text{positive control}} - \text{Mean}_{\text{negative control}}}$$

A pig was considered seropositive for M. hyopneumoniae if the S/P ratio was  $> 0.4$ .

## Data analysis

Data were cleaned in Excel (Microsoft 2016, Redmond, Washington, USA) and transferred to Stata (Stata/MP-13 StataCorp, College Station, Texas, USA) for analysis. All statistical analyses were conducted only on high seropositivity groups. A mixed-effects multi-level logistic regression method with farrowing source and sow was used to compare IAV and M. hyopneumoniae seropositivity at different stages of production (objective 1). As only three groups were classified as high PRRSV seropositivity, a mixed-effects multi-level logistic regression method with only sow as a random effect and farrowing source as fixed effect was used to compare PRRSV seropositivity at different production stages for objective 1. An additional mixed-effects multi-level logistic regression method with farrowing source, sow, and pig (repeated measurement) as random effects was used to determine the association between seropositivity to different pathogens for the IAV and M. hyopneumoniae models (objective 2), as well as the effects of nursery diet complexity on antibody responses (objective 3). Similar models were prepared for PRRSV with only sow and pig as random effects and farrowing source as fixed effect. While this study was not designed to evaluate risk-factors associated with disease susceptibility, certain parameters were included to control for managerial differences between farms. The independent variables considered in the univariable analyses were Cohort (1/2), nursery diet (HC/LC), production stage (at weaning/end of nursery/end of grower/end of finisher), seropositivity to other pathogens of interest for the present study (yes/no), and farrowing source. These variables were first screened by univariable analysis and considered for inclusion in the final model if  $p < 0.2$ . Models were then built using a manual forward stepwise approach and variables were included in the final model if  $p < 0.05$ .

## Results

### Antibody responses

Overall, 6.6% (144/2182), 44% (964/2180), and 24% (502/2078) of samples from all farms were seropositive for PRRSV, IAV, and *M. hyopneumoniae*, respectively. In total, 618, 618, and 590 pigs were tested twice, 537, 536, and 507 were tested three times, and 409, 408, and 391 were tested four times for PRRSV, IAV, and *M. hyopneumoniae*, respectively. There were 336 pigs tested for all three pathogens at four visits. Of the pigs tested for three pathogens at four visits, 24 (7.1%) were seronegative for all three pathogens throughout production, 165 (49.1%) were seropositive for one at least once over the course of production, 124 (36.9%) were seropositive for two, and 23 (6.9%) were seropositive for all 3. The proportions of pigs that were seropositive at least once over the course of production if tested at 4 visits for PRRSV, IAV, and *M. hyopneumoniae* are shown in Figs. 1, 2, and 3, respectively. PRRSV, IAV, and *M. hyopneumoniae* seropositivity for all pigs was 7.9, 61.0, and 31.1%, respectively, at weaning; 1.1, 30.6, and 21.5% at the end of the nursery stage; 8.2, 37.7, and 18.3% at the end of the grower stage; and 10.8, 48.0, and 25.1% at the end of the finisher stage (Fig. 4). A group was classified as high seropositivity for any pathogen if at least 20% of pigs in that group were seropositive at least once for that pathogen over the course of production. Three groups were classified as high seropositivity for PRRSV (45.7–51.1%), all fourteen groups were high seropositivity for IAV (53.9–98.9%), and seven were high seropositivity for *M. hyopneumoniae* (77.9–100%) (Table 1). All statistical models were constructed using the high seropositivity groups only. There were two groups in this study that were vaccinated for *M. hyopneumoniae*, as determined by the survey distributed to producers, and the corresponding groups were removed from further analyses. The subsequent increases in seropositivity in the remaining groups were therefore assumed to be due to natural infection.

Table 1  
Pig-level seropositivity to PRRSV, IAV, and *M. hyopneumoniae* in 14 groups of pigs.

		Farrowing source								
		Cohort	1	2	3	4	5	6	7	8
Seropositivity (%)	PRRSV	One	0.00	0.00	0.00	2.00	9.80	51.11	0.00	16.67
		Two	3.33		0.00	0.00	0.00	40.43		88.64
	IAV	One	65.22	65.38	81.40	64.00	47.06	44.44	97.30	100.00
		Two	36.67		100.00	96.88	94.00	85.11		97.73
	<i>M. hyopneumoniae</i>	One	86.96*	11.54	0.00	2.00	100.00	2.22	97.30	72.92
		Two	78.95*		2.33	0.00	100.00	12.77		86.21

This image depicts the percentage of individual pigs that were seropositive to porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), or *Mycoplasma hyopneumoniae* in 14 groups of pigs from 8 farrowing sources. Highlighted (grey) cells indicate high seropositivity groups, defined as groups with 20% or greater pig-level seropositivity from weaning to the end of finisher. Seropositivity was determined by commercial ELISA. \*These groups were vaccinated for *M. hyopneumoniae*.

Seropositivity profiles for pigs tested at four production stages for all groups are shown in Table 2. The number of times pigs were seropositive if tested at four visits in all 14 groups are shown in Fig. 5.

Table 2. Seropositivity profiles in pigs tested for PRRSV, IAV, and *M. hyopneumoniae*.

Number (%) of pigs			Production stage (age in days)			
PRRSV	IAV	<i>M. hyo</i>	At weaning (19-33)	End of nursery (52-70)	End of grower (96-115)	End of finisher <sup>A</sup> (130-159)
1 (0.3)	48 (13.2)	10 (2.8)				
0 (0.0)	5 (1.4)	2 (0.6)				
0 (0.0)	24 (6.6)	22 (6.1)				
21 (5.8)	54 (14.9)	34 (9.4)				
0 (0.0)	14 (3.9)	1 (0.3)				
1 (0.3)	19 (5.2)	12 (3.3)				
10 (2.8)	42 (11.6)	12 (3.3)				
0 (0.0)	14 (3.9)	4 (1.1)				
1 (0.3)	8 (2.2)	16 (4.4)				
0 (0.0)	13 (3.6)	4 (1.1)				
0 (0.0)	3 (0.8)	2 (0.6)				
1 (0.3)	11 (3.0)	2 (0.6)				
11 (3.0)	31 (8.5)	5 (1.4)				
2 (0.6)	4 (1.1)	2 (0.6)				
15 (4.1)	11 (3.0)	27 (7.4)				
299 (82.6)	62 (17.1)	188 (51.8)				
362 (100)	363 (100)	343 (100)				
			seropositive	<input checked="" type="checkbox"/>		
			seronegative	<input type="checkbox"/>		

Seropositivity profiles were generated for pigs that were tested at 4 visits. This table depicts the number of pigs that were seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* at each or multiple stage(s) of production (weaning, end of nursery, end of grower, and/or end of finisher), e.g. row 1, with black squares at all 4 timepoints, shows the number and percentage of pigs seropositive at all 4 times for PRRSV (column 1), IAV (column 2) and *M. hyopneumoniae* (column 3), while row 2 shows the number and percentage of pigs seropositive for the first 2 time points and negative for the other 2 time points.

<sup>A</sup> animals at the end of finisher time-point were sampled one-week prior to slaughter.

## Multivariable analyses

PRRSV. On the three high seropositive cohorts, pigs were more likely to be seropositive for PRRSV at weaning ( $p < 0.001$ ), the end of grower ( $p < 0.001$ ), and the end of finisher ( $p < 0.001$ ) compared to the end of nursery. Pigs fed a low complexity nursery diet were more likely to be seropositive than those fed a conventional high complexity diet ( $p < 0.001$ ), and farrowing source also affected the likelihood of seropositivity ( $p = 0.015$ ) (Table 3 & Additional File 1).

**Table 3**  
Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity.

<b>Parameter</b>	<b>Odds ratio</b>	<b>Standard error</b>	<b>95% confidence interval</b>	<b>P</b>
Nursery diet				
HC <sup>A</sup>	Referent	–	–	–
LC <sup>A</sup>	18.33	8.90	7.08–47.45	< 0.001
Farrowing source				
6	Referent	–	–	–
8	15.12	14.33	2.36–96.91	0.004

This table displays the mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity with sow and pig as random effects in 3 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

IAV. Pigs were more likely to be seropositive for IAV at weaning ( $p < 0.001$ ), the end of grower ( $p = 0.003$ ), and the end of finisher ( $p < 0.001$ ) compared to the end of nursery. Cohort two pigs were more likely to be seropositive than cohort one ( $p < 0.001$ ) (Table 4). Pigs fed a low complexity nursery diet were more likely to be seropositive than conventionally fed pigs ( $p = 0.04$ ) (Additional File 2). The variation in seropositivity due to the farrowing source and pig was 32 and 68%, respectively.

**Table 4**  
Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity.

<b>Parameter</b>	<b>Odds ratio</b>	<b>Standard error</b>	<b>95% confidence interval</b>	<b>P</b>
Nursery diet				
HC <sup>A</sup>	Referent	–	–	–
LC <sup>A</sup>	1.34	0.19	1.01–1.77	0.04
Cohort				
One	Referent	–	–	–
Two	12.28	4.25	6.23–24.18	< 0.001

This table displays the mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity with farm, sow, and pig as random effects in 14 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

M. hyopneumoniae. Pigs were more likely to be seropositive for M. hyopneumoniae at weaning compared to end of nursery ( $p < 0.001$ ). Pigs that were seropositive for PRRSV were more likely to be seropositive for M. hyopneumoniae ( $p < 0.001$ ), while pigs in cohort two were less likely to be seropositive ( $p < 0.001$ ) (Table 5 and Additional File 3). The variation in seropositivity due to farrowing source and pig was 33 and 67%, respectively.

Table 5  
Mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae*.

Parameter	Odds ratio	Standard error	95% confidence interval	P
PRRSV <sup>A</sup> seropositivity				
Seronegative	Referent	–	–	–
Seropositive	11.43	11.24	1.66–78.59	0.013
Cohort				
One	Referent	–	–	–
Two	0.12	0.08	0.03–0.45	0.002

This table displays the mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity with farm, sow, and pig as random effects in 5 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

## Discussion

This study aimed to investigate antibody responses to porcine reproductive and respiratory syndrome virus, influenza A virus, and *Mycoplasma hyopneumoniae* in pigs at different stages of production, to determine the interaction in antibody response between those pathogens, and to examine the effects of nursery diet complexity on antibody responses to those pathogens.

In general, seropositivity indicates that an animal has either absorbed maternally derived antibodies or been exposed to infectious agents through natural infection or vaccination. In this study, seropositivity proportions were high at weaning for all three pathogens, likely due to the absorption of antibodies through the sow's colostrum and milk (17,18), low at weaning, and high again for PRRSV and IAV at the end of the grower and finisher stages. The decline in seropositivity observed from weaning to nursery in this study indicates the loss of maternal antibodies (2), which also suggests that pigs may be particularly susceptible to pathogens post-weaning. Antibodies to both PRRSV and IAV appeared to be fairly prevalent in the high seropositivity groups, and thus the risk for infection from these agents is high throughout production. Because none of the groups included in the multivariable analyses were vaccinated for any of the pathogens of interest, it is largely assumed that increases in antibody responses post-weaning were the result of natural infection. Implementation of vaccination paradigms may be beneficial to enhance the development of the immune response. Additionally, monitoring for the presence of disease using techniques such as ELISA will help to identify specific pathogens present on a farm and minimize doubt of falsely diagnosing one disease for another.

Unlike PRRS and IAV, pigs were more likely to be seropositive for *M. hyopneumoniae* only at weaning compared to end of nursery in the high seropositivity groups. These results either indicate the vulnerability of weanlings to infection, as antibody responses did not seem to increase significantly in later stages of production, or the lack of *M. hyopneumoniae* infections in these stages. Due to the nature of *M. hyopneumoniae* and its tendency to produce chronic infections in the host, the assumption is that after the decline of maternal antibodies, the young pigs mount a slower immune response (19). This seems to suggest that the pathogen is not being cleared from production but rather the immune response is slower to respond.

The second objective of this study was to determine if infection with one infectious agent influences the activity of another. It was found that pigs seropositive for PRRSV were more likely to be seropositive for *M. hyopneumoniae*. The present study did not determine if co-infection with PRRSV and *M. hyopneumoniae* produced more severe disease, but these results have been reported in the past (9,20). Additionally, while managerial factors, such as pig density and pig flow, would affect the spread of disease on farm, it is possible that infection with one agent would increase susceptibility to the other agent(s). This suggests that while controlling for the presence of one infectious agent is important, in order to prevent more severe disease, care

should be taken to prevent co-infection as much as possible. Understanding which pathogens are a threat on a farm-specific basis using techniques such as ELISA may help in reducing the detrimental effects of co-infection.

The final objective of this study was to investigate whether nursery diet complexity had an impact on antibody responses to PRRSV, IAV, and *M. hyopneumoniae*. Pigs fed a low complexity nursery diet were more likely to be seropositive for PRRSV and may have been likelier to be seropositive for IAV. However, there was no significant association between nursery diet complexity and *M. hyopneumoniae* seropositivity. The diet complexity has also been previously found to have no effect on antibody responses to *Salmonella* (14). These results may suggest that the low complexity diet increased the susceptibility of pigs to PRRS and influenza viruses but had no effect on susceptibility to *M. hyopneumoniae* and *Salmonella*. Alternatively, these results may indicate that the LC diet elevated the immune response to PRRSV and IAV while having no effect on the immune response to *M. hyopneumoniae* and *Salmonella*. Interestingly, nursery diet complexity only affected seropositivity to the viral pathogens but not the bacterial pathogens. The association found between nursery diet and antibody response to pathogens tested in this study should be interpreted with caution and need to be investigated more thoroughly while evaluating innate and cell mediated immune responses in additional pigs. Further investigation into the effects of nursery diet complexity on antibody responses to other notable porcine pathogens may help shed more light on the effects of diet on immune development. Additionally, other branches of the immune system, such as cell-mediated immune responses, could also be examined to determine if additional facets of the immune system are affected.

While seropositivity at the pig level was relatively high, a proportion of pigs remained seronegative throughout all stages of production. This indicates either that these pigs were never exposed to the infectious agents; that animals were exposed but the pathogens were unable to bypass the innate immune system in order to establish infection and activate the adaptive immune system; that an immune response was generated but was not robust enough to be read as seropositive by the ELISA kits; or that pigs had not yet seroconverted at the time of sample collection. However, there may have also been some variation in results based on the ELISA kits used for antibody detection. The IDEXX ELISA kits have been found to have 100% sensitivity and 99.9% specificity for PRRSV (21); 86 and 89% for IAV (22); and relatively low sensitivity (63%) but high specificity (100%) for *M. hyopneumoniae* (19). However, as noted by Erlandson and colleagues (18), the low sensitivity of the *M. hyopneumoniae* test is likely due to the nature of the infectious agent and the slow immune response produced by *M. hyopneumoniae* rather than the efficacy of the ELISA kits themselves. As such, there may have been false negatives generated in this study, but the classification of groups as high and low seropositivity and considering a pig "seropositive" if it tested seropositive at least once over the course of production likely worked to counteract this issue.

The ELISA kits used in this study were unable to differentiate between antibody responses to natural infections and vaccination. However, because only one farrowing source was vaccinated for *M. hyopneumoniae* and the corresponding groups were not included in the analyses, the seropositivity observed in the high seropositivity groups can be largely assumed to be from maternal antibodies in the early stages of production and natural infection later in life. These results may help to encourage vaccination in post-weaning pigs, when the interaction between maternal antibodies and vaccine antigens is minimized (2).

## Conclusion

Understanding periods of vulnerability on farm is important for producers to be able to develop site-specific methods of disease prevention and control. Monitoring frequently for changes in the current pathogenic threats to a farm may help to confer broader protection, improve animal health and welfare, and increase producer profits, as well as ensure animals are not incorrectly treated for a different but clinically similar disease. Finally, while further research is needed to investigate the association between other components of immune system, such as innate and cell mediated immune responses, and low complexity nursery diets and the effect on disease susceptibility, this study suggests low complexity nursery diets, which offer cost-saving incentives, may be beneficial on farms with low disease pressures.

## Declarations

## Ethics approval

Animal use was approved by the University of Guelph Animal Care Committee.

## Consent for publication

Not applicable.

## Availability of data and materials

The data used and analyzed during the current study are available from the corresponding author upon reasonable request.

## Competing interests

The authors declare that they have no competing interests.

## Funding

Funding was provided by NSERC, OMAFRA, Swine Innovation Porc, the Canadian Centre for Swine Improvement, Alliance Genetics Canada, and Ontario Pork.

## Authors Contributions

BNL, AF, and RMF designed the study and provided the laboratory and resources. ER conducted experiments, collected, analyzed, and interpreted the data, and wrote the manuscript.

## Acknowledgements

Thank you to Corinne H. Schut, Pauldeep S. Virk, Bailey M. Fuller, and Dr. Jutta Hammermüller for lab assistance.

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## Additional Files

Additional File 1.doc

Additional File 1. Mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity.

This table displays the mixed-effects multi-level logistic regression analysis for porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity with sow as random effect in 3 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

Additional File 2.doc

Additional File 2. Mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity.

This table displays the mixed-effects multi-level logistic regression analysis for influenza A virus (IAV) seropositivity with farrowing source and sow as random effects in 14 high seropositivity groups. Seropositivity was determined by commercial ELISA.

<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

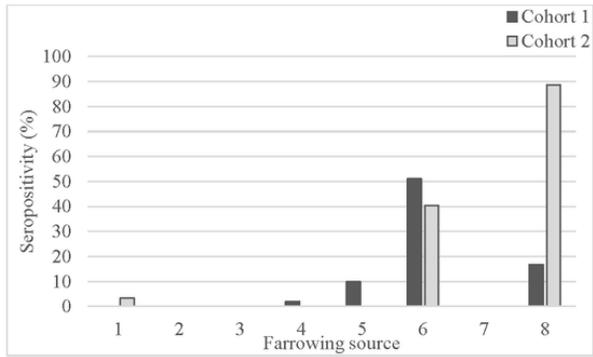
Additional File 3.doc

Additional File 3. Mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity.

This table displays the mixed-effects multi-level logistic regression analysis for *Mycoplasma hyopneumoniae* seropositivity with farrowing source and sow as random effects in 5 high seropositivity groups. Seropositivity was determined by commercial ELISA.

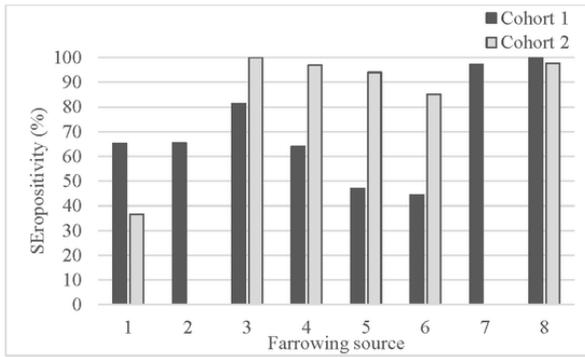
<sup>A</sup>HC = high complexity diet, LC = low complexity diet.

## Figures



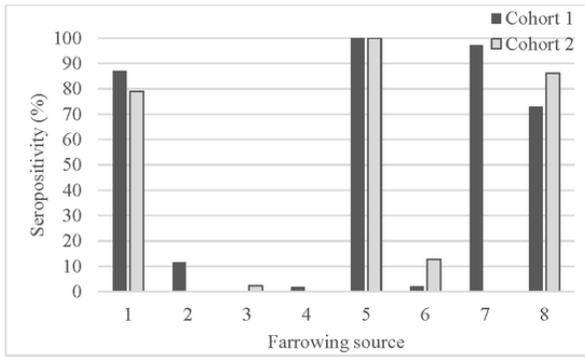
**Figure 1**

Percentage of pigs seropositive for PRRSV at least once from weaning to end of finisher. This figure depicts the percentage of pigs in 14 groups that tested seropositive for porcine reproductive and respiratory syndrome virus (PRRSV) at least once from weaning to the end of the finisher stage. Note: farrowing sources 2 and 7 included only one cohort. Seropositivity was determined by commercial ELISA.



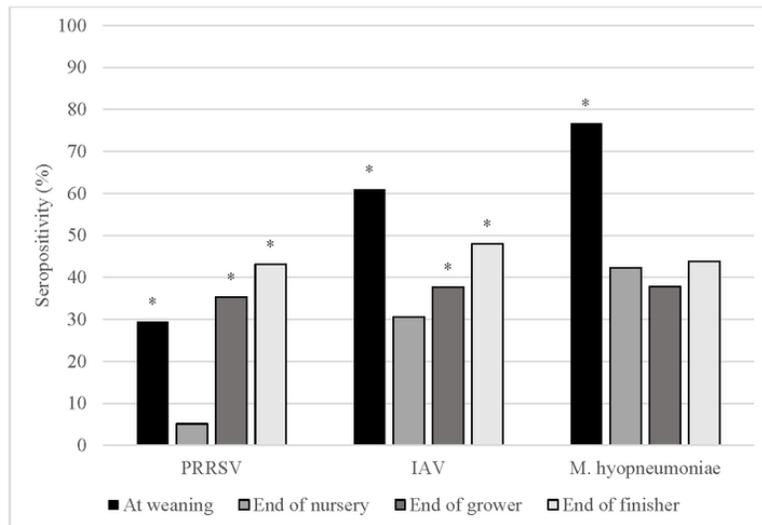
**Figure 2**

Percentage of pigs seropositive for IAV at least once from weaning to end of finisher. This figure depicts the percentage of pigs in 14 groups that tested seropositive for influenza A virus (IAV) at least once from weaning to the end of the finisher stage. Note: farrowing sources 2 and 7 included only one cohort. Seropositivity was determined by commercial ELISA.



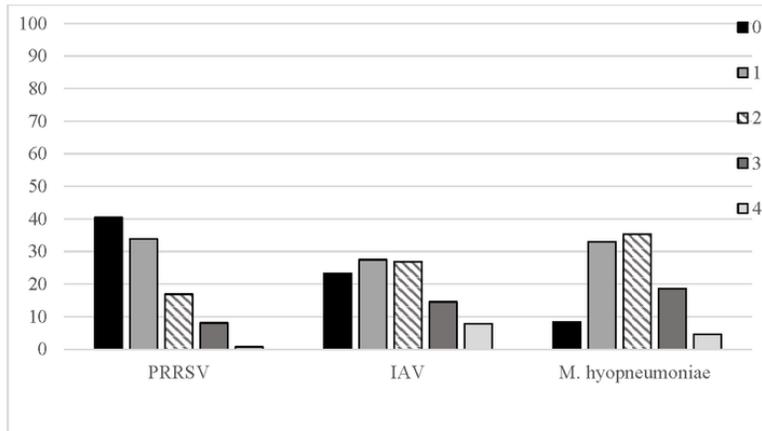
**Figure 3**

Percentage of pigs seropositive for *M. hyopneumoniae* at least once from weaning to end of finisher. This figure depicts the percentage of pigs in 14 groups that tested seropositive for *Mycoplasma hyopneumoniae* at least once from weaning to the end of the finisher stage. Note: farrowing sources 2 and 7 included only one cohort. Farrowing source 1 vaccinated for *M. hyopneumoniae*. Seropositivity was determined by commercial ELISA.



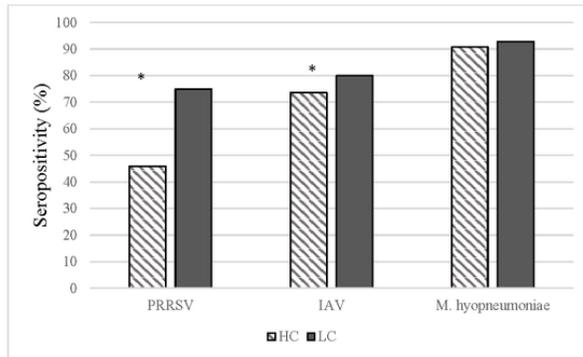
**Figure 4**

Percentage of pigs seropositive for PRRSV, IAV, and M. hyopneumoniae from weaning to the end of finisher. This figure depicts the percentage of pigs seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and Mycoplasma hyopneumoniae in high seropositivity groups at each stage of production from weaning to the end of the finisher stage. Note: Three, 14, and 5 groups were classified as high seropositive for PRRSV, IAV, and M. hyopneumoniae, respectively. Seropositivity was determined by commercial ELISA. \*Significantly different from weaning ( $p < 0.05$ ).



**Figure 5**

Number of times pigs tested seropositive for PRRSV, IAV, and M. hyopneumoniae. This figure depicts the number of times individual pigs tested seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and Mycoplasma hyopneumoniae in high seropositivity groups of pigs from weaning to the end of the finisher stage. Note: Three, 14, and 5 groups were classified as high seropositive. Seropositivity was determined by commercial ELISA.



**Figure 6**

PRRSV, IAV, and *M. hyopneumoniae* seropositivity in pigs fed conventional or low complexity nursery diets. This figure depicts the percentage of pigs seropositive for porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), and *Mycoplasma hyopneumoniae* in high seropositivity groups of pigs fed high complexity (i.e., high animal protein content) or low complexity (i.e., mainly plant-based) nursery diets. Note: Three, 14, and 5 groups were classified as high seropositive. Seropositivity was determined by commercial ELISA. \* $p < 0.05$

## Supplementary Files

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