The Effect of Vaccination of Gilts with a Pre-Farrow RNA Particle Vaccine following Pre-Breeding Natural Planned Exposure on Piglet Growth and Viral Shedding

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Summary and Implications

The objective of this study was to compare rotavirus shedding and performance of piglets from gilts immunized using natural planned exposure (NPE) or an RNA particle vaccine (RPV) prior to farrowing following initial NPE pre-breeding. A final total of 117 farrowed gilts and their piglets were enrolled into 4 groups. All gilts received two administrations of NPE prior to breeding. Gilts in group 1 were later given three NPE administrations at 5, 4, and 3 weeks prior to farrowing (WPF). Group 2 was dosed with an RNA particle vaccine (RPV) at 5 and 3 WPF and group 3 at 1 WPF only. Group 4 (control group) did not receive any NPE or RPV. Fecal samples from gilts and fecal swabs from their piglets were tested for rotavirus A (RVA), rotavirus B (RVB), and rotavirus C (RVC) by qRT-PCR. The 117 gilt samples were tested individually at 5 sampling points from pre-breeding to entry into the farrowing rooms. Piglet samples were pooled by litter (3 piglets sampled per litter) and tested at 3, 7, 14, and 21 days of age. The clinical and production impact of the treatments were assessed by comparing average adjusted weaning weights, the percentage of piglets weighing in the bottom 10% of study piglets at weaning, the percentage of piglets placed in the nursery, and the percentage of litters with the presence of diarrhea. No statistically significant differences were identified but there appeared to be several interesting numerical trends that are both biologically plausible and consistent among treatment groups. The control group, which received no pre-farrow immunization, had the highest percentage of litters with diarrhea at all time points and overall in the farrowing house. A numerical trend was also observed on average cycle threshold (Ct) values for RVC in piglets. Interestingly, the control group had the lowest numerical average Ct value at 7, 14, and 21 days of age. Ct values for RVA were consistently lower than for RVC in the stock solution and natural planned exposure gruel. This likely correlated to a lower level of RVC exposure in all gilts pre-breeding and group 1 gilts pre-farrow. This may have an inverse correlation to the higher levels of shedding of RVC in piglets. The lack of statistical significance between treatment groups with regard to diarrhea and weights in the piglets may be attributed to sample size, lack of sufficient natural rotavirus challenge, pre-breeding NPE in all gilts, or a variety of other reasons. Replication of this study with a larger sample size, using a challenge model, or relocated to a farm with increased natural rotavirus clinical challenges may yield different results. If beneficial under different conditions, the RPV would eliminate many of the risks associated with NPE administration, including continuous introduction of live virus on-farm, potential spread of other pathogens, difficulty of isolating on-farm strains, and labor and cost of producing NPE material. Limitations of the original research include the use of quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) to measure the levels of RVA and RVC in the stock solution and gruel and to monitor RV shedding, rather than techniques with the ability to differentiate live and non-viable virus. Additionally, these studies were performed on an all-gilt breeding farm. It may be more challenging to appreciate differences in treatment groups on a multiparous sow farm due to the higher level of immunity correlated to increasing parity. These immunization protocols were designed to be applicable in a field setting for routine rotavirus immunization, though their feasibility must be carefully considered before implementation on each individual farm.

Introduction

Rotavirus is a common cause of scours in suckling and nursery swine. Young pigs are most often clinically affected by RVA and RVC serogroups, while RVB infections are often less severe.1 The prevalence of RVA and RVC has been recorded at 64% and 58%, respectively.2 Providing protection in neonates and growing pigs is difficult due to

genetic variability of the virus, which has been shown to correlate with limited cross-protection between and within each of the common serogroups. Additionally, RVB and RVC cannot be reliably grown in cell culture, resulting in a lack of vaccines and diagnostic tools. Therefore, NPE is widely used to immunize sows prior to farrowing to promote the transfer of passive immunity to their piglets. A previous study showed that NPE at 5, 4, and 3 WPF resulted in a 1.4-pound increase in average pig weaning weight on the study farm.3 Despite the success of feedback on many swine farms, there are still concerns regarding compliance and administration techniques, inconsistencies in viral dose, and potential introduction of other harmful pathogens inadvertently. Furthermore, Anderson et al (2022) showed that some gilts were still shedding rotavirus when they entered the farrowing house, potentially exposing their piglets to higher levels of rotavirus.3 In contrast, control gilts that did not receive NPE were not shedding RVs at the time of farrowing. These findings contributed to a desire to booster RV immunity without introducing live virus to animals through feeding intestines and feces. Vaccination of gilts pre-farrow with an RNA particle vaccine (RPV) after initial live virus exposure prior to breeding is one possible way to limit live virus feedback.

This study compares two RPV protocols to NPE at 5, 4, and 3 WPF, which was the most successful of the studied protocols in the previous trial. The objective was to compare RV shedding and performance of piglets from gilts immunized using NPE or RPV prior to farrowing following initial NPE pre-breeding.

Materials and Methods

The swine used in this study were cared for in adherence with Pork Quality Assurance Plus (PQA Plus) guidelines. The gilts and piglets in this study were housed at an 1,800-head commercial swine farm in the United States. The breeding population was made up of primarily bred gilts that transferred to a different farm after weaning their first litter. Two breeding groups (approximately 200 gilts) were allotted into four treatment groups. This was done using a random number generator with stratification by expected farrowing date. All groups received two doses of NPE prior to breeding in the farm's on-site gilt development unit (GDU). The gilts were housed in stalls until pregnancy testing and were then moved to pens for the remainder of gestation. Gilts were penned with other gilts of their same treatment group to allow for the proper administration of NPE and RPV pre-farrow. Treatment group 1 received NPE at 5, 4, and 3 weeks pre-farrow (WPF). This protocol was shown to yield increased wean weights in piglets compared to those from gilts that did not receive NPE. Treatment group 2 was injected with a rotavirus RNA particle vaccine (RPV) at 5 and 2 WPF. The timing of injections for this group is consistent with the label recommendation for Prosystem RCE (Merck Animal Health), which is commonly used in the US swine industry. Treatment group 3 was injected with RPV at 1 WPF only. This protocol was chosen to assess the efficacy of rotavirus immunization at a typical timepoint for PEDV vaccination in the industry to maximize labor efficiency. Although the study farm was negative for PEDV, this may be advantageous for other farms. The control group did not receive any NPE or RPV pre-farrow. All NPE and RPV administrations are depicted in table 1 and were supervised by the primary investigators. All animals in the same breeding group were administered their treatment at one time point, which led to a range of actual intervals in each group once farrowing occurred. However, due to the potential effects of induction on piglet weight and health, induction was performed. The time of administration prior to farrowing was based on the average farrowing date of the group with regard to farrowing due dates. For example, the time of vaccination for 2 weeks prior to farrowing ranged from 11 to 17 days prior to the due date. Group 3 gilts were vaccinated at least 7 days prior to farrowing. These administration times were chosen to ensure adequate response time post-administration while maintaining applicability to commercial farms.

Gilts were loaded into the farrowing rooms several days prior to their expected farrowing date. They were each assigned farrowing stalls to minimize environmental variation for piglets. Four adjacent farrowing crates were identified as a block. Within each block, one gilt from each treatment group was placed randomly.

Litters were officially enrolled for data collection in the study if they were farrowed within a period of ten days. Litters from gilts that were farrowed outside of the study window were ineligible for final selection, resulting in a total of 126 eligible litters. Of these litters, nine were removed for the following reasons; three due to cross-fostering of non-study piglets into the litter, five because less than seven piglets were born alive, one due to an injury to the sow, and one as a consequence of agalactia. In total, sampling and data collection was performed on 117 litters. Cross fostering was prohibited for these enrolled litters. At two days of age, all piglets from all enrolled litters were individually weighed and tagged. Tags indicated a unique identification number to facilitate later sampling of individual pigs. The piglet weights were separated into tertiles within their litter (heaviest, middle, and lightest). One pig per weight tertile was randomly selected for sampling using a random number generator, yielding three pigs to sample per litter. Pre-determined alternates were identified in case of mortality of selected piglets.

NPE material was developed with farm-specific RVA and RVC using the master seed method.4 The master seed method was developed to minimize inconsistencies in the viral dose of NPE and prevent contamination of feedback with other pathogens. The RVs on the farm were previously monitored with qRT-PCR and sequence analysis of VP4 and VP7. RVB was not routinely identified on the study farm and therefore was not included in the NPE material. Two

NPE administrations were given to all gilts within 3 weeks of breeding at a 4-day interval in the on-site GDU.

The NPE mixture was prepared by adding 40 mL of master seed to enough water and feed to prepare 100 doses of gruel, as described more thoroughly in Anderson et al (2022).3 One dose of NPE was provided to each gilt, and researchers remained nearby to confirm that each dose was completely consumed. At each NPE administration, gruel samples were kept for testing of RVs by qRT-PCR. The NPE administrations were performed by the primary investigators to ensure proper dosage.

The product used for this study was manufactured by Sequivity (Merck Animal Health), utilizing an RNA (ribonucleic acid) particle technology to produce the vaccine.5 The vaccine included two RVA replicons and one RVC replicon that matched the isolates used in the pre-breeding and pre-farrow NPE. Testing prior to vaccination via qRT-PCR and sequencing on piglet feces confirmed that these subtypes were still present in the herd. RPV development begins with the sequencing of target genes from farm-specific pathogens. The genes are then amplified by PCR and cloned into a replicon vector, resulting in the production of RNA transcripts. The next step includes pulsation of RNA replicons and helper RNAs with Vero cells in an electroporation chamber, followed by incubation for 18 hours. The final vaccine is prepared after lysing of the cells in the culture fluid.6 Gilts in treatment groups 2 and 3 received one or more doses of RPV according to the protocols explained above. Vaccination was performed by injecting 1 mL of RPV intramuscularly.

Fecal samples were collected from gilts prior to pre-breeding exposure, 1 week after pre-breeding exposures, at 5 WPF, at 4 WPF, and after loading gilts into the farrowing house. These samples were selected to confirm proper NPE administration and monitor RV shedding at critical time points, such as entry into farrowing. Gilt fecal samples were collected by manual stimulation and stored in 50-mL centrifuge tubes labeled with the gilt's tag number. Fecal swabs were collected from the previously selected three piglets per litter at 3, 7, 14, and 21 days of age. A pre-packaged virus transport system (BD BBL™ CultureSwab™) with a swab, transport media and transport tube was used for collecting piglet fecal samples. The tag number and sampling point were recorded on each tube. All samples were stored at -80 °C until testing. In addition, to sample collection for diagnostic testing, subjective litter diarrhea observations were recorded weekly. The litter was recorded as having diarrhea present if any loose stools were observed. If all feces present in the farrowing crate at the time of observation were within normal expectations for piglet stools, they were recorded as not having diarrhea. The same investigator observed and recorded the presence or absence of diarrhea at each time point and was blinded to the treatment groups. Weights were recorded for all pigs in every enrolled litter, not just the selected three for sampling, at two days of age and three days prior to weaning.

Gilt fecal samples, piglet fecal swabs, and NPE gruel samples were tested by qRT-PCR for RVA, RVB, and RVC. Piglet samples were pooled by litter, while gilt samples were tested individually. Diagnostic testing was completed at the Kansas State University Veterinary Diagnostic Laboratory.

Biosecurity was a high priority while executing this trial. Inter litter biosecurity was maintained as thoroughly as possible by prohibiting entering the crate by the farm staff and researchers, changing gloves between litters when handling the pigs, and disinfecting instruments and scales after each litter. Clinical diarrhea scouring was limited to visual observation that did not require handling the piglets. Farm staff and researchers were blinded to the treatment groups while taking observations, sampling, and weighing.

Piglet weights were collected twice, as specified above. Production data, including pre-weaning mortality, was recorded via the farm's record-keeping system (PigKnows). Clinical data was also collected by observing the presence or absence of diarrhea in each crate at 3 time points (1 week old, 2 weeks of age, and prior to weaning).

Generalized linear (mixed effects) models were used to explain the effect of treatment on several response variables, including piglet weaning weight, percentage of piglets in the bottom 10% of weaning weights, percentage of piglets placed in the nursery, and percentage of scouring litters.

Treatment was included as a fixed effect for all four response variables, and the gilt was included as a random effect for the first three. Models were fit via PROC GLIMMIX. All least squares means were reported, along with all pairwise differences of least squares means. Statistical significance was set at $p < 0.05$.

Weaning weights were adjusted to a 21-day estimation by adding or subtracting 0.5 lbs for each day of age under or over 21 days of age when weighed, respectively.7 Additionally, piglets in the bottom 10% based on weaning weight were identified for analysis by treatment group. This was done to determine if any group had a disproportionate amount of light pigs since weaned pig weight and cost per pound of weaned pig are important metrics in some pork production companies. Production data was recorded using the farm's record-keeping system (PigKnows). Production records were cross-referenced with the information on each sow card to ensure accuracy.

Results and Discussion

The clinical and production impact of the treatments were assessed by comparing average adjusted weaning weights, the percentage of piglets weighing in the bottom 10% of study piglets at weaning, the percentage of piglets placed in the nursery, and the percentage of litters with the presence of diarrhea.

The average adjusted weaning weights for each group are shown in figure 1. There were no statistically significant differences in adjusted weaning weights between the treatment groups. With respect to the analysis on piglets

weighing in the bottom 10% of weaning weights, there were also no statistically significant differences between treatment groups. The percentages of pigs in each treatment group that fell into this category are shown in figure 2. Numerically, treatment group 2 had the highest percentage of light weaned pigs at 10.91%. The percentage of pigs born alive that were placed in the nursery was also analyzed as a measure of survivability and quality. Piglets not placed in the nursery were either pre-weaning mortalities or pigs euthanized due to poor viability. These results are found in figure 3. Treatment group 1 had the highest percentage of pigs placed in the nursery at 86.47%; however, there were no statistically significant differences.

Litters were observed for the presence of diarrhea at 3 time points, including 1 week old, 2 weeks of age, and prior to weaning (figure 4). All treatment groups had the highest prevalence of observable diarrhea at one week of age. Levels appeared to decrease at two weeks of age but increased again near the time of weaning. The control group numerically had the highest percentage of litters with diarrhea at all time points. Treatment group 1 had the next highest percentage of scouring litters overall. Despite numerical differences, there were no statistically significant differences in diarrhea prevalence.

Rotavirus stock samples and mixed gruel samples were tested by qRT-PCR for RVA, RVB, and RVC at all administration time points. All samples were negative for RVB. As expected, the stock material yielded lower cycle threshold (Ct) values for RVA and RVC compared to the gruel at all administrations (Table 2). The Ct values for RVA were lower than RVC in all samples, indicating a higher RVA concentration in the NPE material. RVA virus isolation was not completed for each sample, due to previous validation of viable virus in the samples.

Gilts were fecal sampled prior to pre-breeding NPE, after pre-breeding NPE, at 5 WPF, at 4 WPF, and at the time of entry into the farrowing house. At the fourth sampling point (4 WPF), only gilts in treatment group 1 were sampled since they were the only group receiving NPE pre-farrow. Fecal samples were tested for RVA, RVB, and RVC. The percentages of positive gilts for RVA and RVC in each treatment group at each sampling point are found in figures 5 and 6. There were very few RVB positive samples, which were not reported due to their irrelevance to this study. There was a low level of RVA shedding in gilts prior to the pre-breeding NPE administrations; however, RVC was not detected in any of the gilt fecal samples at this time. After pre-breeding NPE administration, just over half of the trial gilts shed RVA with variations in the percentage in each treatment group. Conversely, only three total gilt fecal samples were positive for RVC at this time point. Treatment group 1 gilts, which were administered NPE pre-farrow, were then sampled at 4 WPF. This resulted in shedding of RVA in 90.3% of gilts, while only 25.8% were positive for RVC. At farrowing, RVA and RVC shedding was at or below 10% in all treatment groups. Numerically, RVA shedding was highest in the control group, while all control gilts were negative for RVC at this time.

Figures 7 and 8 show the average cycle threshold (Ct) values for RVA and RVC positive gilts at each sampling point for each treatment group. No identifiable trends are evident in these figures; however, they do provide insight into the levels of RVs shed in adult animals.

Piglets were fecal swabbed at four sampling points, including 3, 7, 14, and 21 days of age. Fecal swabs were tested for RVA, RVB, and RVC. Shedding of RVA in piglets was not observed at any sampling point in any treatment groups. However, RVC was shed at every time point in all treatment groups. The percentages of positive litters for RVC in each treatment group at each sampling point are found in figure 9. At 3 days of age, just one litter in each treatment group was shedding RVC. However, at 7 and 14 days of age, treatment group 1 had the highest levels of RVC shedding numerically. Among all of the groups, the lowest level of RVC shedding was at 3 days of age. Shedding increased at each sampling point, with the highest levels of RVC being shed at 21 days of age.

Figure 10 shows the average cycle threshold (Ct) values for RVC positive litters at each sampling point for each treatment group. Interestingly, the control group had the lowest numerical average Ct value at 7, 14, and 21 days of age. The largest variation in average Ct values occurred at 3 days of age, while there was only a small range of average Ct values at 14 and 21 days of age.

While no differences achieved the a priori threshold for statistical significance, there appear to be several interesting numerical trends that are both biologically plausible and consistent among treatment groups. Firstly, the control group, which received no pre-farrow immunization, had the highest percentage of litters with diarrhea at all time points and overall in the farrowing house. The next highest group with respect to the percentage of scouring litters in the farrowing house at two of the 3 time points and overall was treatment group 1 (NPE at 5, 4, and 3 WPF). It is possible that there was a clinical advantage to rotavirus immunization via RPV, seeing that less diarrhea was observed in the treatment groups that received RPV. Another observation was that Ct values for RVA were consistently lower than for RVC in the stock solution and natural planned exposure gruel. This likely correlated to a lower level of RVC exposure in all gilts pre-breeding and group 1 gilts pre-farrow. Additionally, the percentage of gilts shedding RVC pre-breeding and pre-farrow was much lower than that for RVA. This may have an inverse correlation to the higher levels of shedding of RVC in piglets. A numerical trend was also observed on average Ct values for RVC in piglets. Interestingly, the control group had the lowest numerical average Ct value at 7, 14, and 21 days of age. This is consistent with a lack of pre-farrow immunization in gilts that may result in less lactogenic antibodies secreted to piglets, worsened clinical rotavirus disease, and increased shedding.

The lack of statistical significance between treatment groups with regard to diarrhea and weights in the piglets may be attributed to a low level of exposure in the farrowing room. The farm on which the study was conducted has strictly adhered to an NPE program for an extended period of time. It is possible that this immunization program reduced the environment RV load that piglets are challenged with over time. Since an intentional RV challenge was not incorporated in the study design, treatment group differences were reliant on sufficient natural RV exposure.

This study also differed from previously conducted trials through the addition of a pre-breeding NPE administration. The investigators implemented a pre-breeding administration to ensure all gilts had a sufficient live RV challenge prior to being vaccinated. Research has shown that non-live vaccinations are much more effective after animals have experienced a previous live pathogen exposure.8 Although this pre-breeding exposure was thought to be essential to the study, it is possible that the NPE provided an adequate level of immunity in all treatment groups that lasted until the time of farrowing.

Additional limitations of this study include the use of qRT-PCR to measure the level of RVA and RVC in the stock solution and gruel and to monitor RV shedding. Due to the nature of this diagnostic test, it cannot be guaranteed that the virus was viable. Virus isolation could have been performed but was not practical due to the size of the study and intent to keep protocols feasible for field implementation. In addition, the implementation of NPE and RPV may be difficult or prohibited depending on the farm characteristics and relevant regional regulations.

With an understanding of the limitations of this study, it would not be appropriate to rule out RPV as a potential immunization method. It may be valuable on farms with lower levels of RV immunity and clinical RV challenges. If efficacious, the RPV would eliminate many of the risks associated with NPE administration, including the continuous introduction of live virus on-farm, the potential spread of other pathogens, the difficulty of isolating on-farm strains, and labor and cost of producing NPE material.

Under the conditions of this study there were no statistically significant differences between treatment groups, though there were several numeric trends suggesting RPV may still have potential for RV immunization under different conditions. RVC exposure and shedding in gilts was lower than that for RVA, which may inversely correlate to the higher RVC shedding observed in piglets. The control group numerically had the highest percentage of scouring litters in the farrowing house and the lowest average Ct values. Further research or a larger study is needed to elucidate the impact of RNA particle vaccine technology on farms with lower RV immunity and/or significant RV challenge to piglets.

Acknowledgements

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Table 1. Natural planned exposure (NPE) and RNA particle vaccine (RPV) administration protocols by treatment group

Treatment Group	Pre-breeding (4-day interval)		Pre-farrowing				
			5 wks	4 wks	3 wks	2 wks	1 wk
1	NPE	NPE	NPE	NPE	NPE	-	-
2	NPE	NPE	RPV	-	-	RPV	-
3	NPE	NPE	-	-	-	-	RPV
Control	NPE	NPE	-	-	-	-	-

Table 2. Cycle threshold (Ct) values from rotavirus A (RVA) and rotavirus C (RVC) quantitative reverse transcriptase-polymerase chain reaction testing of stock and gruel mixtures at each administration

	Stock RVA	Gruel RVA	Stock RVC	Gruel RVC
Pre-Breeding #1*	12.9	22.4	18.0	27.2
Pre-Breeding #2*	13.6	25.4	19.5	29.8
5 Weeks Pre-Farrow†	15.9	25.0	21.0	29.1
4 Weeks Pre-Farrow†	14.3	24.2	19.4	27.8
3 Weeks Pre-Farrow†	14.0	25.2	20.4	30.0
Range	3.0	3.0	3.0	2.8

*Time points at which natural planned exposure was administered to all enrolled gilts
†Time points at which natural planned exposure was only administered to treatment group 1

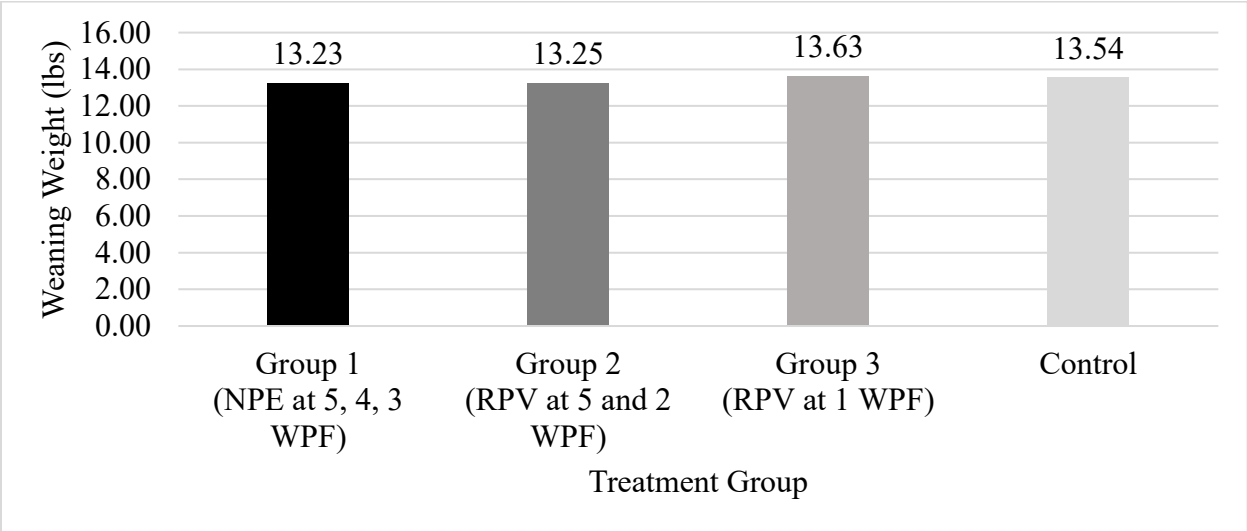

Figure 1. Average adjusted piglet weaning weights in pounds for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF). No statistically significant differences were identified between treatments groups at $p < 0.05$.

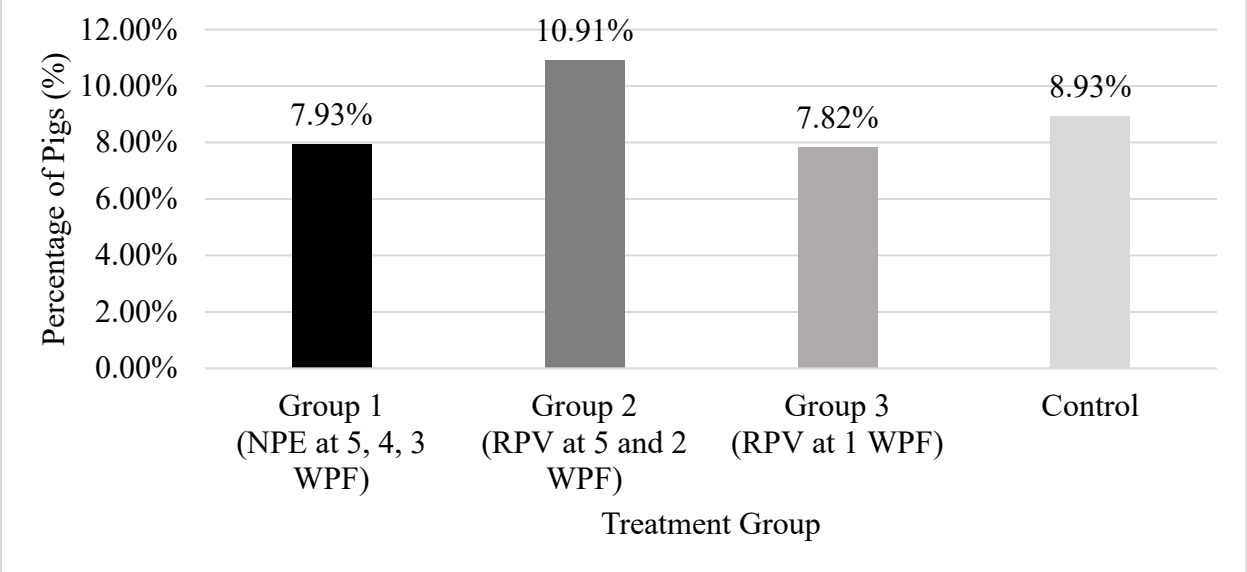

Figure 2. Percentage of pigs in the bottom 10% of trial pig weaning weights for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF). No statistically significant differences were identified between treatments groups at $p < 0.05$.

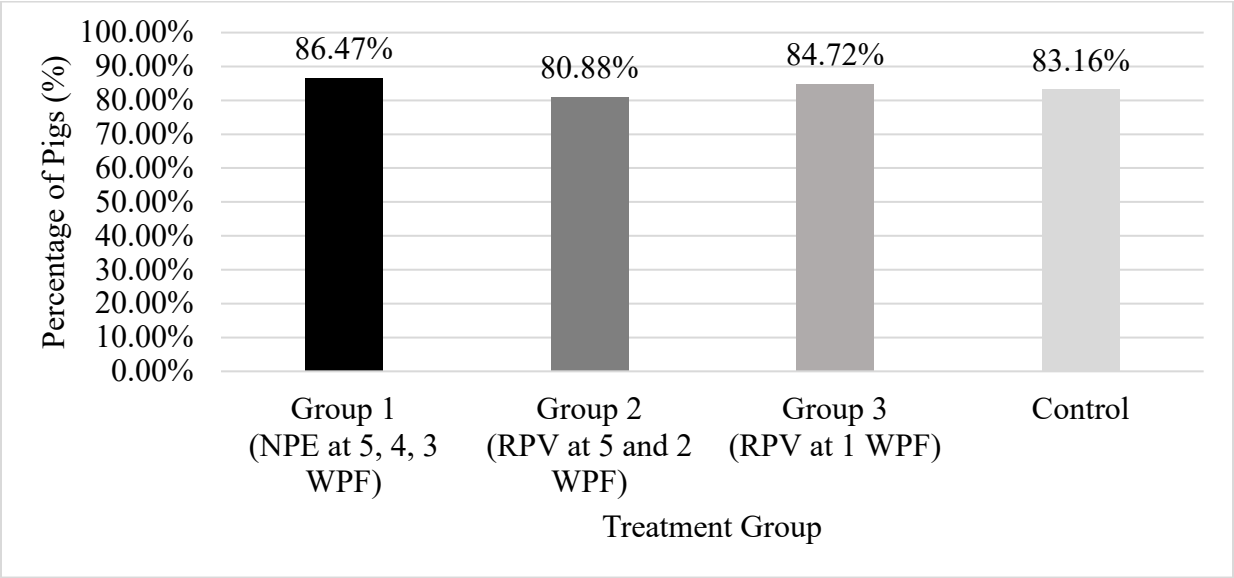

Figure 3. Percentage of pigs placed in the nursery for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF). No statistically significant differences were identified between treatments groups at $p < 0.05$.

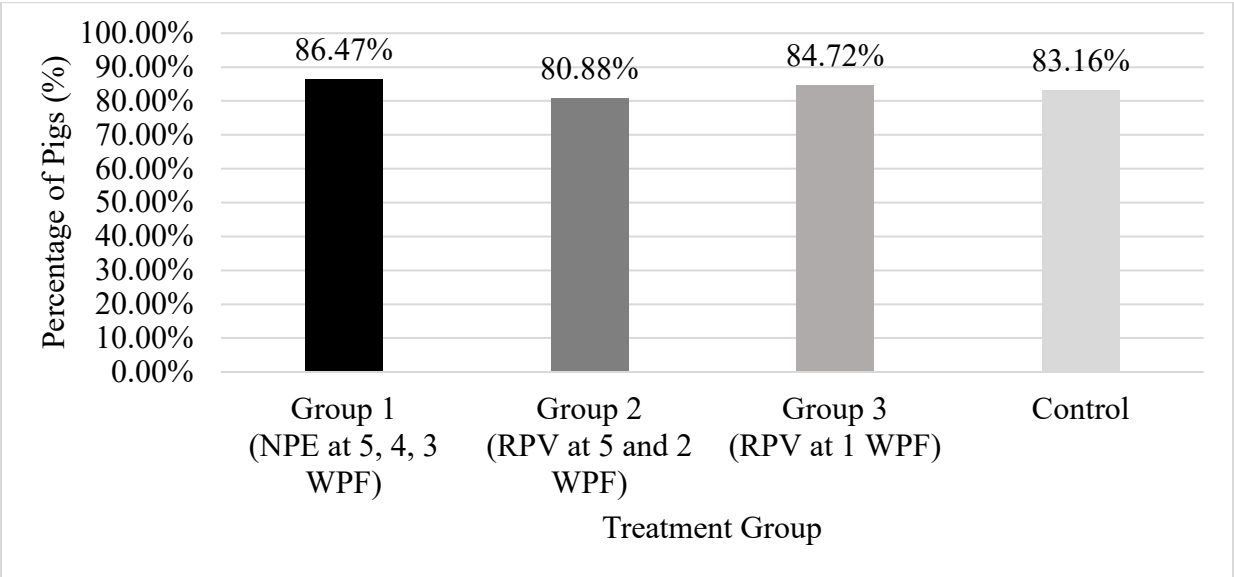

Figure 4. Percentage of litters with diarrhea at 3 time points and overall in the farrowing house for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF). No statistically significant differences were identified between treatments groups at $p < 0.05$.

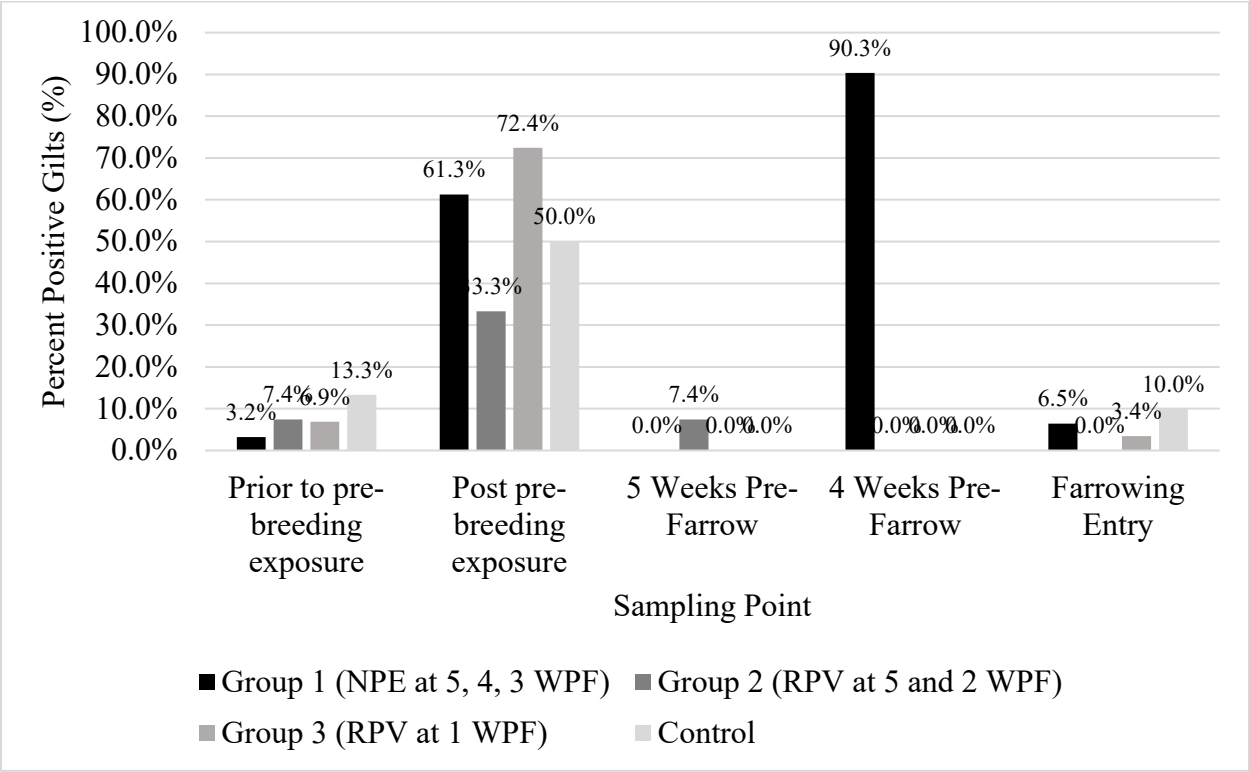

Figure 5. Percentage of gilts positive for rotavirus A (RVA) for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF). No statistically significant differences were identified between treatments groups at $p < 0.05$.

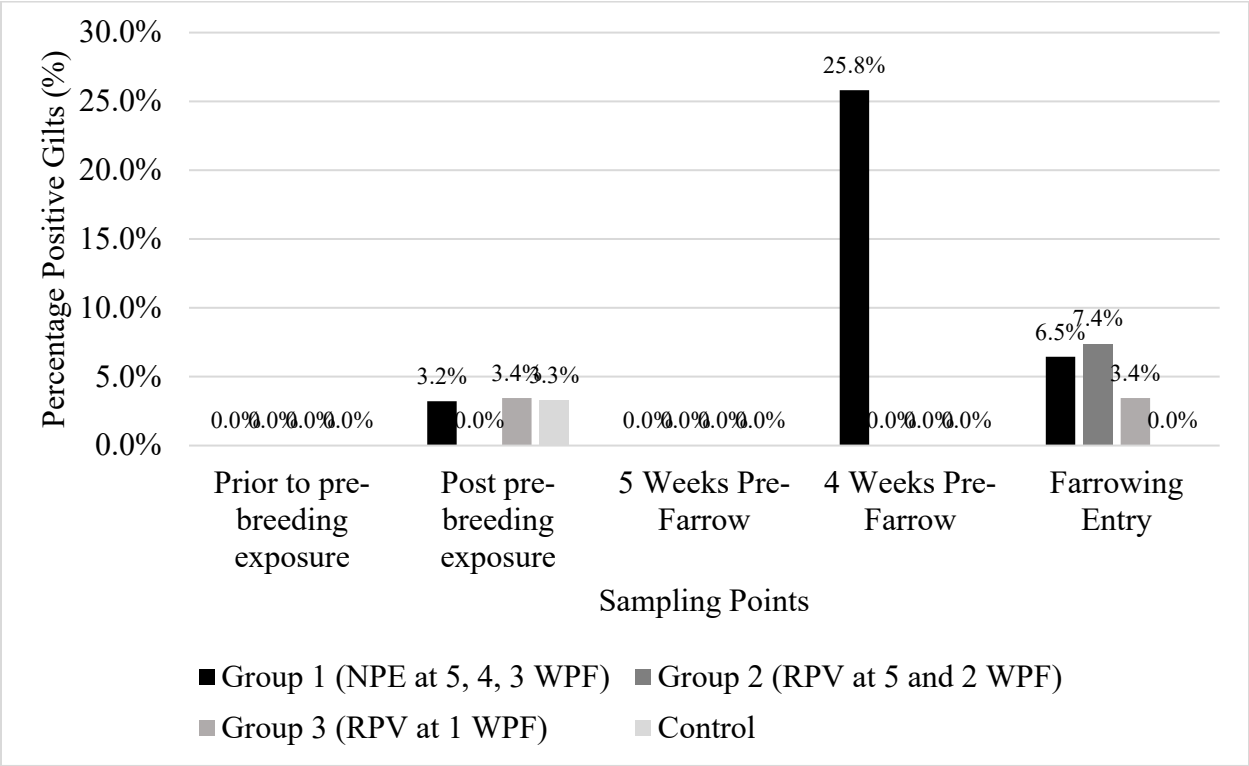

Figure 6. Percentage of positive gilts for rotavirus C (RVC) for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF).

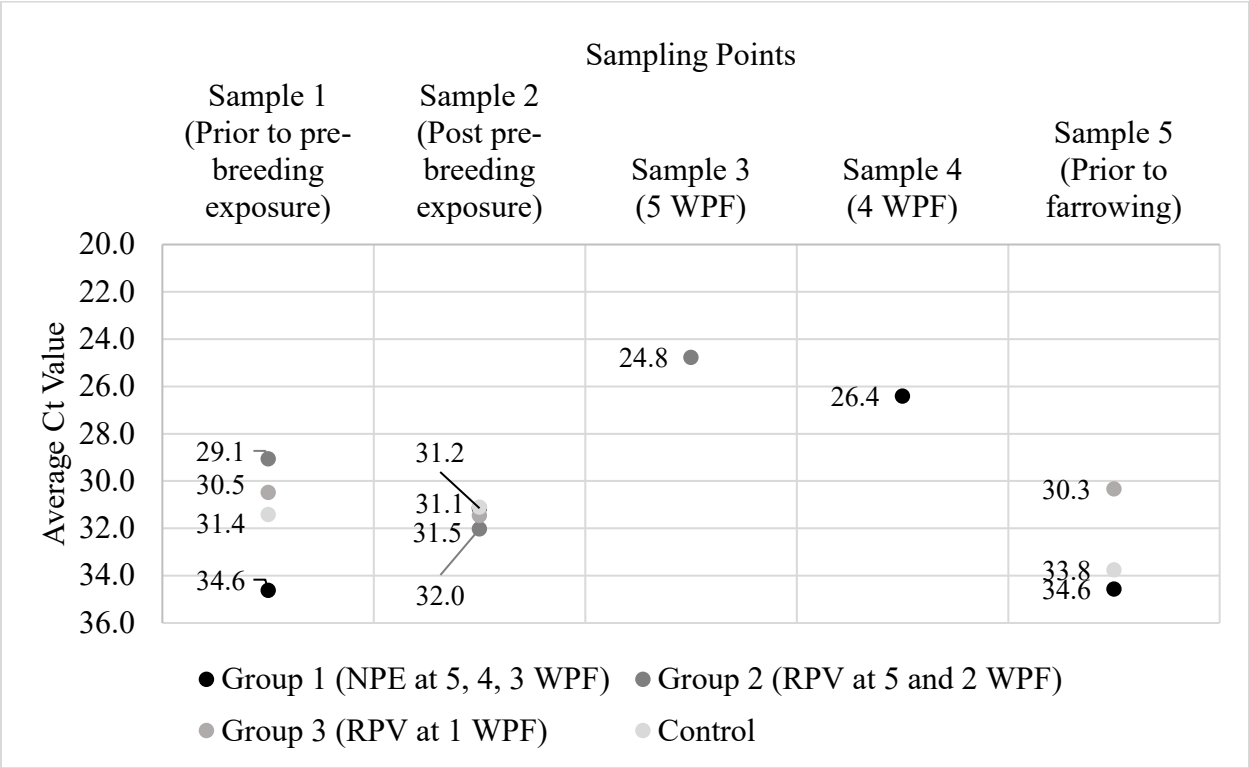

Figure 7. Rotavirus A (RVA) average cycle threshold (Ct) value of positive gilts by quantitative reverse transcription-polymerase chain reaction for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF).

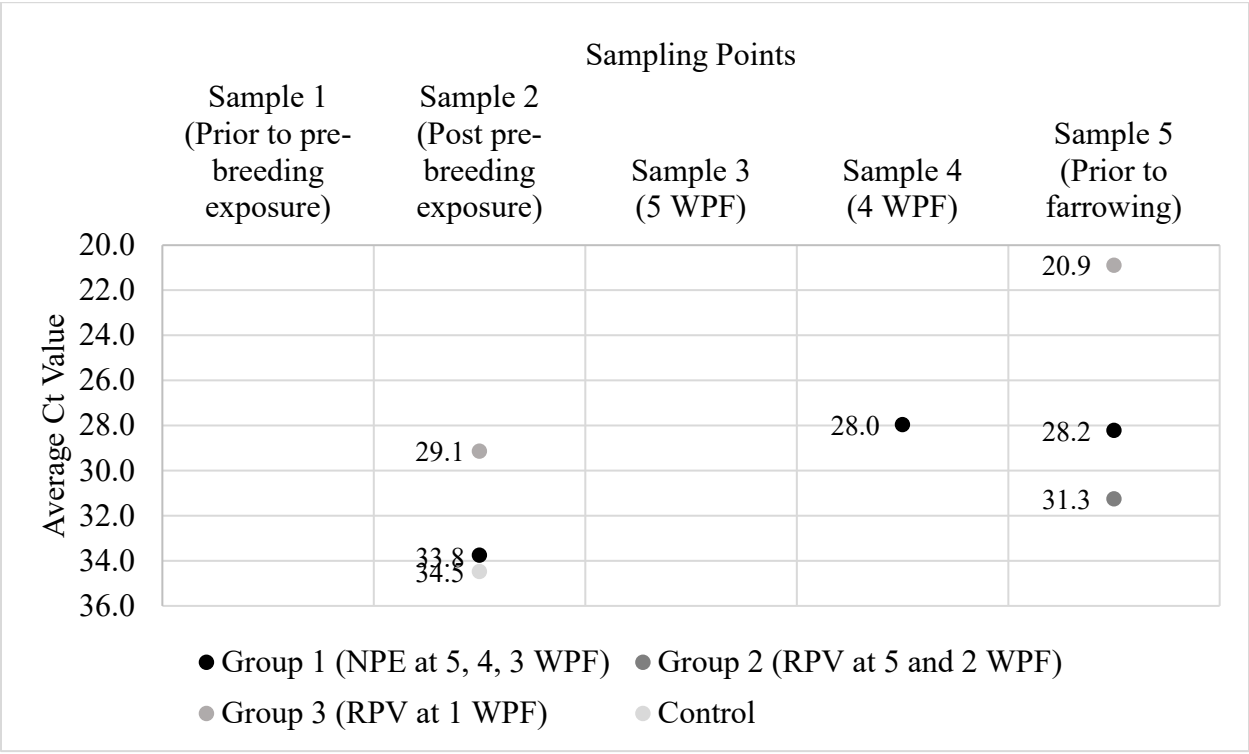

Figure 8. Rotavirus C (RVC) average cycle threshold (Ct) value of positive gilts by quantitative reverse transcription-polymerase chain reaction for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF).

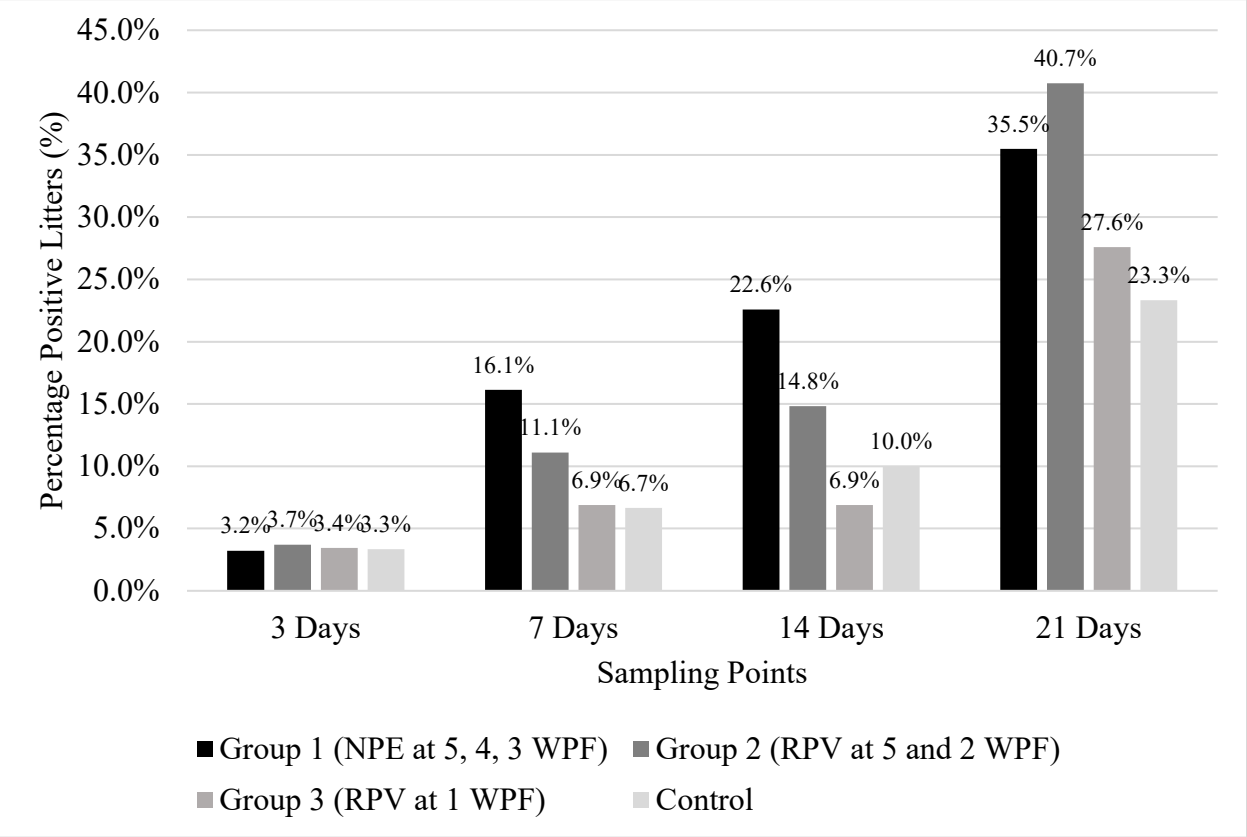

Figure 9. Rotavirus C (RVC) average cycle threshold (Ct) value of positive gilts by quantitative reverse transcription-polymerase chain reaction for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF).

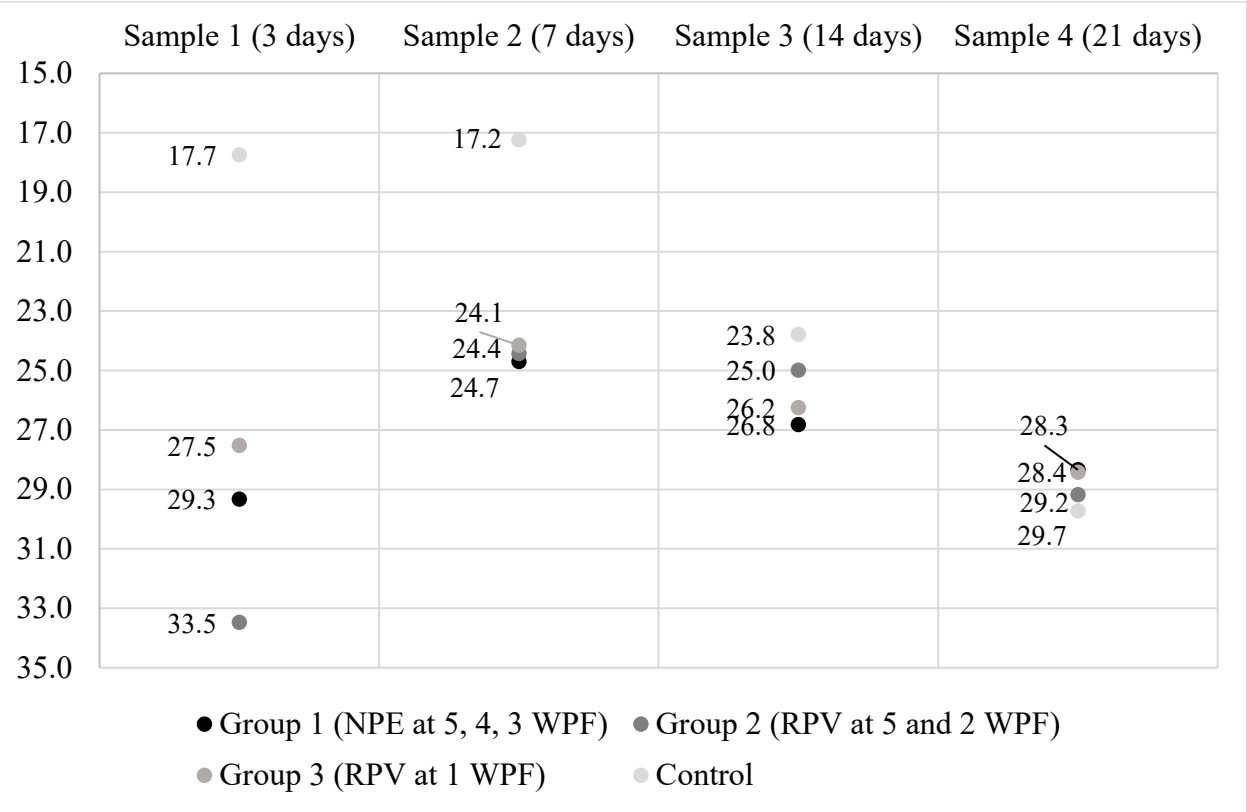

Figure 10. Rotavirus C (RVC) average cycle threshold (Ct) value of positive litters by quantitative reverse transcription-polymerase chain reaction at each sampling point for each treatment group administered natural planned exposure (NPE), RNA particle vaccination (RPV), or no immunization at several time points in weeks pre-farrow (WPF).