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Detection and Investigation of Atypical Porcine Pestivirus within a Breed-to-Finish Farm and Off-Site Nursery and Finisher Locations

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Detection and Investigation of Atypical Porcine Pestivirus within a Breed-to-Finish Farm and Off-Site Nursery and Finisher Locations

Abstract

Atypical porcine pestivirus (APPV) has been associated with congenital tremors (CT) and splay leg (SL) in piglets of infected dams. The major cost of this virus is the increased pre-weaning mortality due to CT or SL interfering with the piglet's ability to nurse and move around the farrowing stall. A commercial farrow-to-finish farm with replacement gilts coming from an off-site genetic multiplier farm, and semen delivery from a commercial boar stud began to see an increase of CT and SL in the farrowing room in early 2020. Diagnostics on clinically affected pigs' samples identified APPV RNA and no other suspected pathogen. At this point, the origin of the virus and means of introduction into the farm was unknown since the farm had no previous clinical cases of CT or SL prior to this investigation. The two hypothesized routes were the introduction of replacement gilts or incoming semen doses. Therefore, the objectives of this investigation were to determine the prevalence of clinical APPV cases at the farrow-to-finish farm, understand the route of introduction of APPV into the farrowto- finish farm, and understand the prevalence of APPV viremia within a population of offspring from a gilt multiplication farm through an off-site nursery and finisher barn. Farrowing records from the farm were analyzed for the presence of CT or SL and parities of females with affected litters. Blood samples were collected at two different times from the new group of replacement gilts and maternal barrows at the isolation nursery barn. Serum and oral fluids were collected from the same pigs at an off-site finisher barn to determine APPV persistence. The APPV sequencing was conducted on a serum sample from a gilt housed at the isolation nursery intended as a replacement gilt for the farrow-to-finish farm, semen dose utilized at the farrow-to-finish farm, and serum of a clinically affected piglet in the farrowing room of the farrow-to-finish farm. Overall, the prevalence of affected litters within batch farrowing groups ranged from 0 to 31%. The prevalence of APPV within samples pooled by pens (5 pigs) ranged from 37.5 to 77.5%, while individual prevalence ranged from 20 to 40%. When followed to the finisher, the same group of pigs had an APPV prevalence in serum ranging from 0 to 26%, while oral fluid prevalence was 100%. Sequencing results indicated that the virus circulating in clinically affected piglets was the most similar to an incoming semen dose. In summary, introduction of APPV into a naïve herd is associated with an increase in clinical CT and SL. While APPV is present in herds previously exposed to APPV, the APPV RNA remains detectable in serum and oral fluids with no clinical disease. To decrease the chance of infection to a naïve herd, quarantines should be implemented for all introductions. Additionally, semen should be screened for APPV presence if there is a recent onset of clinically affected piglets with CT or SL with no other explanation. The APPV RNA was detected in group oral fluids, suggesting the technique may be used to screen incoming animals.

Keywords

Atypical porcine pestivirus, finisher, nursery, over time prevalence

Authors

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KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2021

Detection and Investigation of Atypical Porcine Pestivirus within a Breed-to-Finish Farm and Off-Site Nursery and Finisher Locations

C. Grace Elijah, Cassandra K. Jones, Jason C. Woodworth, Rachel Palinski,[1] Chad B. Paulk,[2] Tom Petznick,[3] and Jordan T. Gebhardt[1]

Summary

Atypical porcine pestivirus (APPV) has been associated with congenital tremors (CT) and splay leg (SL) in piglets of infected dams. The major cost of this virus is the increased pre-weaning mortality due to CT or SL interfering with the piglet's ability to nurse and move around the farrowing stall. A commercial farrow-to-finish farm with replacement gilts coming from an off-site genetic multiplier farm, and semen delivery from a commercial boar stud began to see an increase of CT and SL in the farrowing room in early 2020. Diagnostics on clinically affected pigs' samples identified APPV RNA and no other suspected pathogen. At this point, the origin of the virus and means of introduction into the farm was unknown since the farm had no previous clinical cases of CT or SL prior to this investigation. The two hypothesized routes were the introduction of replacement gilts or incoming semen doses. Therefore, the objectives of this investigation were to determine the prevalence of clinical APPV cases at the farrow-to-finish farm, understand the route of introduction of APPV into the farrow-to-finish farm, and understand the prevalence of APPV viremia within a population of offspring from a gilt multiplication farm through an off-site nursery and finisher barn. Farrowing records from the farm were analyzed for the presence of CT or SL and parities of females with affected litters. Blood samples were collected at two different times from the new group of replacement gilts and maternal barrows at the isolation nursery barn. Serum and oral fluids were collected from the same pigs at an off-site finisher barn to determine APPV persistence. The APPV sequencing was conducted on a serum sample from a gilt housed at the isolation nursery intended as a replacement gilt for the farrow-to-finish farm, semen dose utilized at the farrow-to-finish farm, and serum of a clinically affected piglet in the farrowing room of the farrow-to-finish farm. Overall, the prevalence of affected litters within batch farrowing groups ranged from 0 to 31%. The prevalence of APPV within samples pooled by pens (5 pigs) ranged from 37.5 to 77.5%, while individual prevalence ranged from 20 to 40%. When followed to the finisher, the

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same group of pigs had an APPV prevalence in serum ranging from 0 to 26%, while oral fluid prevalence was 100%. Sequencing results indicated that the virus circulating in clinically affected piglets was the most similar to an incoming semen dose. In summary, introduction of APPV into a naïve herd is associated with an increase in clinical CT and SL. While APPV is present in herds previously exposed to APPV, the APPV RNA remains detectable in serum and oral fluids with no clinical disease. To decrease the chance of infection to a naïve herd, quarantines should be implemented for all introductions. Additionally, semen should be screened for APPV presence if there is a recent onset of clinically affected piglets with CT or SL with no other explanation. The APPV RNA was detected in group oral fluids, suggesting the technique may be used to screen incoming animals.

Introduction

Atypical porcine pestivirus (APPV) is part of the *Flaviviridae* family and has recently been associated with clinical signs of congenital tremor (CT) and splay leg (SL) in piglets during the lactation phase. Clinical signs occur in piglets exposed to the virus during gestation when the dam is viremic for APPV prior to day 70 of gestation.[4] The major cost associated with APPV is pre-weaning mortality due to inability to nurse or move about the farrowing stall.[5] These clinical signs are usually most prominent during lactation, while clinical signs become less common after weaning. This study's objective was to determine the prevalence of clinical cases of APPV in a naïve farrow to finish swine farm, understand the route of introduction, and assess the prevalence of APPV within a population of nursery and finishing pigs.

Materials and Methods

Case history

The commercial 150 sow farrow-to-finish facility utilizes a batch farrow system (approximately 30 sows/batch farrowing every 35 days) with all pigs raised in the on-site nursery and grow-finish facilities. Females are kept in stalls until confirmed pregnant at approximately d 30 of gestation, then introduced into group housing for the remainder of gestation. Females are brought into the farrowing room approximately 3 to 7 d prior to the expected farrowing date. Each farrowing batch has a mix of gilts and sows. In early 2020, clinical CT and SL piglets were observed in the farrowing room. Serum samples were submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) for clinical diagnosis. The serum samples were confirmed positive by APPV qRT-PCR. The two suspected routes of APPV entry into the farm were by replacement gilts or semen doses. Two randomly selected semen doses were submitted to the KSVDL for APPV qRT-PCR, and one of the two doses had detectable APPV RNA.

The isolation nursery receives 400 pigs with 370 to 380 maternal barrows and 20 to 30 replacement gilts approximately every 2 months from a gilt multiplication farm. At the end of every isolation nursery group, the gilts are transported to the farrow-to-

[4] Arruda BL, Arruda PH, Magstadt DR, Schwartz KJ, Dohlman T, Schleining JA, et al. (2016) Identification of a divergent lineage porcine pestivirus in nursing piglets with congenital tremors and reproduction of disease following experimental inoculation. PLoS ONE 11(2): e150104. doi:10.1371/journal.pone.0150104.

[5] Garro, IRH, Sonalio K, and de Oliveria LG (2019) Atypical porcine pestivirus (APPV) as a new species of pestivirus in pig production. Front. Vet. Sci. 6:35. doi:10.3389/fvets.2019.00035.

finish farm after confirmation of PRRSV-negative ELISA and PCR assays. This is the only link the farrow-to-finish farm has with this isolation nursery. The barrows in this nursery are shipped to an off-site finisher facility at the end of each nursery group. In this isolation nursery, piglets are split between two barns with forty pens in each barn and five piglets placed in each pen. Since this isolation nursery supplies gilts to the farrow-to-finish farm at the end of every turn, five random pigs were selected during the August 2020 turn to evaluate for prevalence of APPV. These piglets were bled and serum was submitted to the KSVDL for APPV qRT-PCR. Two of the piglets were confirmed positive for APPV. These results indicate that some piglets were APPV RNAemic at time of placement in the isolation nursery. RNAemia indicates that RNA of the virus was found in the serum allowing for the conclusion that the virus is actively circulating in the animal at that given sample date. Following this test, the entire barn had serum collected and tested for APPV. No animals housed in the isolation nursery had clinical signs of APPV.

Farrowing data record analysis

The farrow-to-finish site's farrowing records from 1/10/2019 until 3/2/2021 were analyzed for the prevalence of litters displaying signs of CT or SL. If a comment of "shakers" or "splays" was written on the farrowing card by the farrowing attendant, the litter was counted in the litter prevalence calculation and divided by the total number of females that farrowed during the specified batch. Parity information was also taken from the same farrowing cards. If the farrowing card indicated the parity of the female as "1" these females were included in the "parity 1" group and designated as gilts, while all else parities were specified as "parity >= 2" and designated as sows. To determine prevalence based on parity, females were sorted into groups based on parity number then divided by the total number of litters with clinically affected piglets per farrowing group.

Blood and oral fluids collection

Serum from the maternal barrows was collected a total of four times—twice in the isolation nursery barn and twice in the off-site finisher barn. Oral fluids for the same barrows were collected twice during the finisher stage. While barrows were housed in the isolation nursery barn, serum was collected on 8/20/2020 and 9/30/2020. On 8/20/2020, 200 maternal barrows were individually bled for serum. For this blood collection date, serum samples were pooled and submitted by pen (5 pigs/pen). The same process was done on 9/30/2020 but a total of 160 pigs were individually bled. The 40 pigs which were not able to be sampled had been removed from the facility as part of a concurrent research trial. Prevalence was calculated for each bleeding date by dividing the total number of pens that had a qRT-PCR positive reaction for APPV RNA by the total number of pens. Aliquots of individual pig serum were retained in a -112°F freezer. As the study progressed, a subset of individual pig serum aliquots was defrosted and submitted for APPV qRT-PCR to determine individual pig APPV RNAemia at time of blood collection.

In the off-site finisher barn, blood and oral fluids were collected on 12/3/2020 and 1/16/2021. Each pig was bled with a clean, individual needle and red top tube. Five oral fluid collection cotton ropes were hung in each pen for approximately ten minutes, then saliva was extracted from the rope and stored in a 15 mL conical tube for a total of ten oral fluid samples. Blood samples and oral fluids were submitted to the KSVDL for

APPV qRT-PCR. If individual pigs were positive, the pig was counted then the total of positive pigs was divided by total number of pigs sampled on that sampling date to get prevalence for APPV viremia for sampling day. If oral fluids had detectable APPV RNA, the rope placement locations were counted and divided by the total number of ropes for the sampling date.

Sequencing and phylogenetic analysis

Samples submitted for sequencing were confirmed positive for detectable APPV RNA via qRT-PCR at the KSVDL. Three total samples were submitted for APPV E2 gene sequencing – one serum sample from a piglet with cycle threshold (Ct) value of 26.25 from the farrow-to-finish farm, one semen dose intended for artificial insemination of gilts or sows with Ct value of 30.46, and one serum sample from a replacement gilt housed at the isolation nursery to be moved to the farrow-to-finish farm with Ct value of 27.48. Except for the semen dose, which generated a partial E2 sequence, two samples generated complete E2 sequences. Viral RNA was extracted with the MagMax viral RNA Isolation kit (Thermo Fisher) on a Kingfisher platform. Amplicons were generated from viral RNA using Superscript III One-Step RT-PCR System with Platinum Taq and specific primers, using a 133°F annealing temperature and 30 s extension time. Amplicons were purified using the HighPrep PCR Clean-up System, library prepped by Nextera XT v2 DNA Library prep kit and sequenced on an Illumina Iseq (300-cycle cartridge), as specified by the manufacturer. Raw reads were trimmed for quality and mapped to the closest Genbank reference (UNL082017 #MK728876). Consensus sequences were extracted from the mapping and used for subsequent analysis. All bioinformatics was performed in CLC workbench v20 using default parameters.

Results and Discussion

For the farm, the prevalence of litters with clinical signs of CT or SL ranged from 0 to 31% within farrowing group (Figure 1). When considering the parity of the litters' dams, clinically affected litters were born to a mix of gilts and multiparous sows (Figure 2). This was unexpected within this investigation as shaker litters are most commonly observed from gilts, and with much less frequency in multiparous sows. Therefore, it was believed in the current investigation that the farrow-to-finish herd was naïve for APPV and an introduction resulted in clinical disease regardless of parity.

Sequencing of three samples revealed that there were two distinct APPV viruses identified at the farrow-to-finish farm (Figure 3), one from the replacement gilts and maternal barrows at the off-site isolation nursery, and the second from piglets and semen dose. The semen dose was 98.9% similar to the clinical piglet, but only 95.2% similar to the gilt sample. Furthermore, the clinical piglet was 95.9% similar to the gilt sample. Thus, based on the evidence in this investigation, it appears that the virus detected in clinically affected piglets was most similar to virus detected in incoming semen.

For the isolation nursery, prevalence for APPV based on the pooled pen samples on 8/20/2020 was 37.5%, then at the end of turn, APPV prevalence from 9/30/2020 was 77.5% (Figure 4). The APPV prevalence in individual pigs on 8/20/2020 was 20%, while prevalence on 9/30/2020 was 40% (Table 1). When these maternal barrows from the isolation nursery were followed to the finisher, there was a 26.7% prevalence for detectable APPV RNA on 12/3/2020 and 0% prevalence on 1/16/2021 (Table 2). Barrows that were positive for APPV were cross referenced to retain individual pig

serum samples from the isolation nursery. All barrows that contained detectable APPV RNA during the finishing period originated from pens in the isolation nursery which did not contain detectable APPV RNA within pooled samples. There was a 100% prevalence in oral fluids for detectable APPV RNA on both sampling dates at the off-site finishing facility.

In summary, when introduced into a naïve herd, APPV can cause clinical signs of CT and SL regardless of parity. In this investigation, it was hypothesized that incoming gilts or semen doses were the most likely routes of introduction, and phylogenetic analysis indicated that the virus circulating in clinically affected piglets shared the highest similarity to the virus detected in an incoming semen dose. The APPV RNA was detected in group oral fluids, suggesting the technique could be used to screen incoming animals.

Table 1. Detectable atypical porcine pestivirus (APPV) RNAemia status for 4 pens in an isolation nursery barn on two separate sampling dates

	Sampling day	
Item	**8/20/2020**	**9/30/2020**
Pen 11		
Pig 173	Positive - 24.38	-
Pig 188	ND	ND
Pig 47	ND	-
Pig 104	ND	Positive - 26.99
Pig 37	ND	-
Pen 16		
Pig 70	ND	-
Pig 110	Positive - 24.66	-
Pig 36	ND	ND
Pig 54	ND	ND
Pig 137	ND	Positive - 26.32
Pen 28		
Pig 96	ND	Positive - 30.46
Pig 123	ND	Positive - 31.78
Pig 105	ND	-
Pig 168	Positive - 30.08	ND
Pig 29	ND	Positive - 35.33
Pen 38		
Pig 181	ND	Positive - 33.14
Pig 184	ND	ND
Pig 182	ND	ND
Pig 69	Positive - 33.01	-
Pig 12	ND	ND

If the individual pig's results had detectable APPV RNA, the cycle threshold value is given after the hyphen.
ND = non-detectable APPV RNA. A dash indicates the individual pig was not present for the second sampling date.

Table 2. Detectable atypical porcine pestivirus (APPV) RNA for 30 individual pig serum samples on different sampling dates

	Sampling day	
Item	**12/3/2020**	**1/16/2021**
Serum		
1	Positive – 36.02	ND
2	Positive – 30.89	ND
3	ND	ND
4	ND	ND
5	ND	ND
6	ND	ND
7	ND	ND
8	ND	ND
9	ND	ND
10	Positive – 35.67	ND
11	ND	ND
12	ND	ND
13	ND	ND
14	ND	ND
15	ND	ND
16	ND	ND
17	ND	ND
18	ND	ND
19	ND	ND
20	ND	ND
21	ND	ND
22	Positive – 34.81	ND
23	ND	ND
24	ND	ND
25	Positive – 36.29	ND
26	Positive – 32.19	ND
27	Positive – 37.24	ND
28	Positive – 35.24	ND
29	ND	ND
30	ND	ND

continued

Table 2. Detectable atypical porcine pestivirus (APPV) RNA for 30 individual pig serum samples on different sampling dates

	Sampling day	
Item	**12/3/2020**	**1/16/2021**
Oral fluid		
1	Positive – 25.62	Positive – 23.36
2	Positive – 21.90	Positive – 25.44
3	Positive – 24.78	Positive – 23.42
4	Positive – 28.06	Positive – 22.06
5	Positive – 25.21	Positive – 28.67
6	Positive – 20.35	Positive – 25.52
7	Positive – 19.80	Positive – 27.66
8	Positive – 20.71	Positive – 24.14
9	Positive – 21.80	Positive – 24.36
10	Positive – 21.68	Positive – 21.40

If the individual pig's results had detectable APPV RNA, the cycle threshold value is given after the hyphen.
ND = non-detectable APPV RNA.

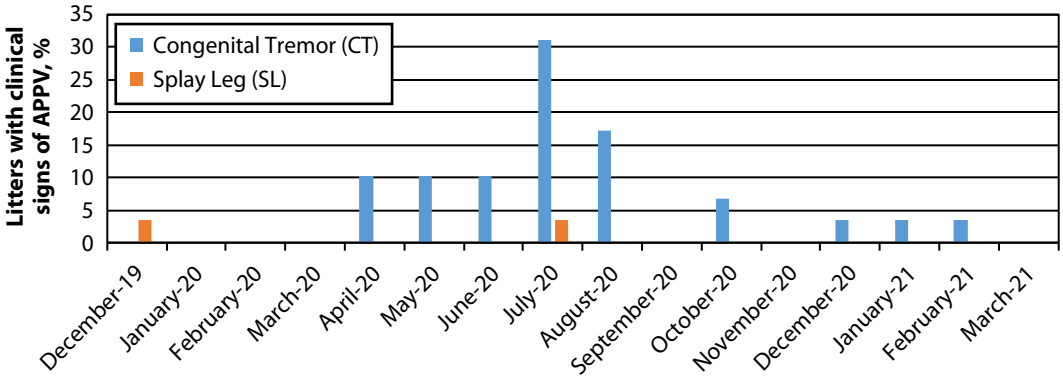

Figure 1. Percentage of litters with either congenital tremors or splay legs over time at a farrow-to-finish farm. Date is specified by month dash year.

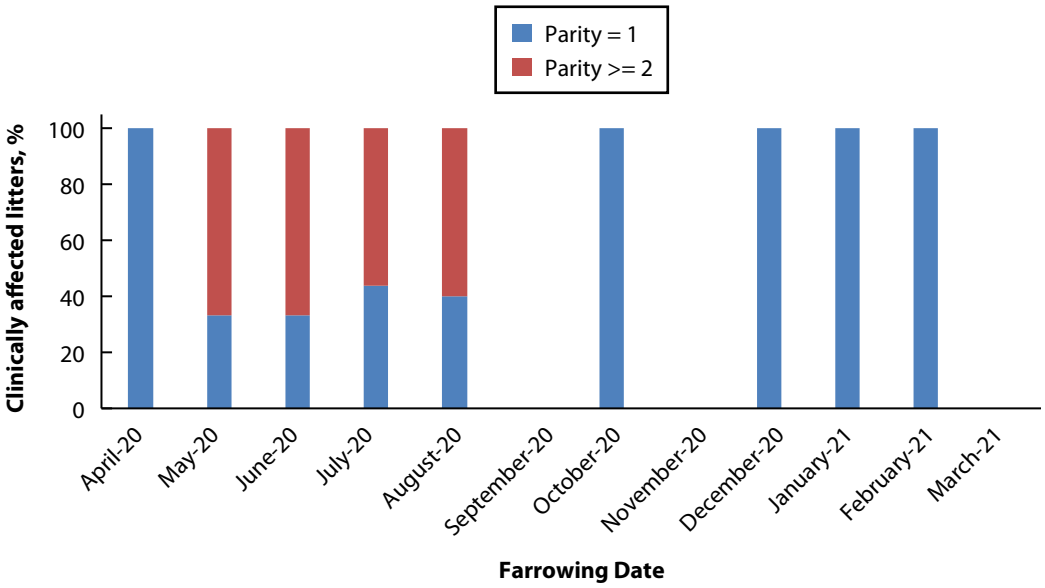

Figure 2. Parity breakdown of litters clinically affected with atypical porcine pestivirus within a farrow-to-finish farm.

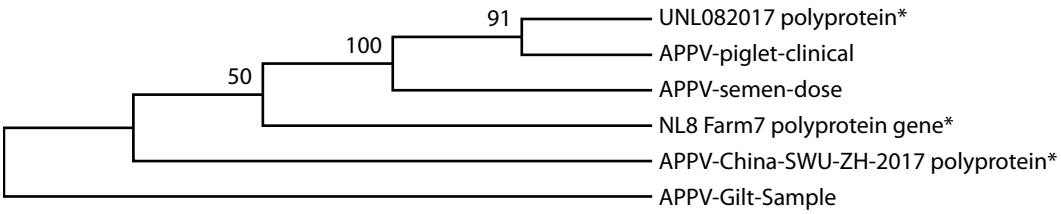

Figure 3. Maximum likelihood phylogenetic tree generated with 1,000 bootstrap replicates (MEGA-X) for atypical porcine pestivirus (APPV) E2; evolutionary history was inferred by using the maximum likelihood method and Tamura-Nei model. Sequences were obtained from a semen dose, serum from a clinically affected piglet, and serum from an incoming gilt (these are named APPV – and designation as to which sample they are). Reference sequences from GenBank are designated with an asterisk (UNL082017 accession number: MK728876.1; NL8 Farm 7 accession number: KX929068.1; APPV-China-SWU-ZH-2017 accession number: H499643.1).

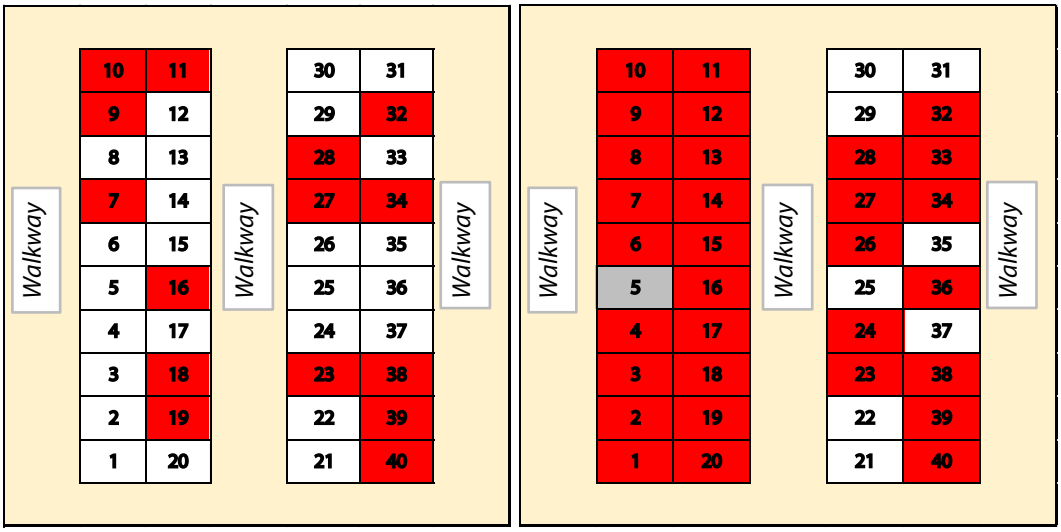

Figure 4. Prevalence of atypical porcine pestivirus (APPV) RNAemia in serum samples pooled by pen in an isolation nursery facility. The diagram on the left is from sampling on 8/20/2020, while the diagram on the right is from sampling on 9/30/2020. Grey indicates a suspected detectable APPV RNA, red pen indicates pooled serum with detectable APPV RNA, and white pen indicates no detectable APPV RNA.