An outbreak of splayleg and congenital tremors in piglets farrowed by a newly populated sow herd

Robert Desrosiers, DVM, DABVP; Élisabeth Carrière, DVM; André Broes, DVM, PhD

Summary

A newly populated sow herd suffered an outbreak of splayleg and congenital tremors in the offspring. Some piglets were affected by one or the other condition, others by both. The problem lasted for about 9 months and was associated with significant losses, mainly because of the splayleg component. Most piglets with only congenital tremors were able to survive and their condition improved as they got older. Piglets with congenital tremors had histological lesions consistent with this condition, and pestivirus K (formerly atypical porcine pestivirus) was identified from their nervous tissues.

Keywords: swine, splayleg, congenital tremors, pestivirus K

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Resumen - Un brote de patas abiertas y temblores congénitos en lechones paridos en una piara de cerdas recién poblada

Una piara de cerdas recién poblada sufrió un brote de patas abiertas y temblores congénitos en las crías. Algunos lechones se vieron afectados por una u otra condición, otros por ambas. El problema duró alrededor de 9 meses y estuvo asociado con pérdidas significativas, principalmente por el componente patas abiertas. La mayoría de los lechones con solo temblores congénitos sobrevivieron y su condición mejoró a medida que aumentaron de edad. Los lechones con temblores congénitos tenían lesiones histológicas compatibles con esta condición y se identificó el pestivirus K, anteriormente llamado pestivirus porcino atípico, a partir de sus tejidos nerviosos.

Résumé - Une épidémie de 'splayleg' (porcelets nageurs) et de tremblements congénitaux chez des porcelets mis bas par un troupeau de truies nouvellement peuplé

Un troupeau de truies nouvellement peuplé a souffert d'une épidémie de splayleg et de tremblements congénitaux chez la progéniture. Certains porcelets étaient atteints de l'une ou l'autre affection, et d'autres des deux. Le problème a duré environ 9 mois et a été associé à des pertes importantes, principalement à cause de la composante splayleg. La plupart des porcelets qui n'avaient que des tremblements congénitaux ont pu survivre et leur état s'est amélioré avec l'âge. Les porcelets atteints de tremblements congénitaux présentaient des lésions histologiques compatibles avec cette affection et le pestivirus K, anciennement appelé pestivirus porcin atypique, a été identifié à partir de leurs tissus nerveux.

The recent identification of pestivirus K (PK), previously known as atypical porcine pestivirus, and piglets born with congenital tremors (CT) after pregnant animals were inoculated with the virus have been major steps in our understanding of this disease.[1-4] Nevertheless there is still limited information concerning the transmission, pathogenesis, carriage, and epidemiology of the virus.[5] Splayleg (SL) is another congenital problem for which questions remain, including possible etiologies. This case report describes an outbreak involving both conditions where losses were significant and lasted longer than what is commonly seen in the field.

Animal care and use

The animals in the case herd were adequately housed, and humanely cared for.

Case description

A 1400-sow herd using a 4-week batch farrowing system was populated in 2019, with the first weaning on December 18. In the first batch, 270 litters were farrowed and many piglets were affected with SL, CT, or both. In that batch, 5.81% (187 piglets) of the total live born pigs were reported to have died because of SL. Most piglets affected with CT appeared to survive. As opposed to SL, mortality records did not include CT as a cause so the exact number of pigs that died because of that condition is unknown. Similarly, the number of pigs affected with both conditions was not compiled. The problems persisted in subsequent batches. When the attending veterinarian visited in June 2020, 7 months after the first litters

presented with these conditions, many litters were still affected. Table 1 summarizes the observations made during that visit. That particular batch had 13.6 liveborn piglets/sow and was weaned on July 1.

There was a total of 259 sows in lactation. A piglet with both conditions was recorded as a CT piglet and an SL piglet. Of the 55 affected litters, 52 were from parity 2 females and 3 were from parity 1 females. In litters with both conditions, the mean number of piglets affected with SL was 2.69 times greater than in litters where only this condition was observed. This increase in dually affected litters was not seen with CT, where the mean number of affected pigs were similar (5.87 and 6.0 piglets/litter, respectively). The mortality associated with SL in that batch was 3.82% (134 of 3511 pigs born live). It decreased further in the next batch (2.32%) and stabilized at about 1% in subsequent batches. Table 2 shows the mortality associated with SL in the first 7 batches following population (December 2019 – June 2020), and in the last 7 batches for which data are available (March 2021 – August 2021).

Two submissions were made to the diagnostic laboratory in January 2020. In the first submission, two 2-week-old piglets with clinical signs of CT were submitted. Histological lesions consistent with CT, including hypomyelination, were observed. A pool of spinal cord samples from both piglets was positive for PK by polymerase chain reaction (PCR) with a cycle threshold value of 28.53. The second laboratory submission included two 4-week-old piglets weaned the week before. One of them showed slight trembling and had histological lesions consistent with CT. A pool of nervous tissue from that piglet also came back positive for PK with a cycle threshold value of 28.41.

Because losses persisted, an attempt was made to inoculate gilts with serum from piglets affected with CT prior to their introduction into the sow herd. Blood was collected from 20 piglets with CT at 2 to 3 days of age, centrifuged, and serum collected and stored at -20°C. Seven of the serum samples were positive for PK by PCR. Serum samples from the 20 piglets were pooled (total of 47 mL). Two 1-mL vials of the pooled sample were sent to Iowa State University Veterinary Diagnostic Laboratory for quantification and came back with cycle threshold values of 34.8 and 33.5. Ninety-seven milliliters of phosphate-buffered saline and 2 mL of ceftiofur (Excenel, Zoetis) were added to the remaining 45 mL of serum for a total volume of 144 mL. Seventy-seven gilts weighing 120 kg were received on July 14. On July 17, 10 gilts were inoculated intramuscularly with a 2 mL dose of the pooled piglet serum. Since there were no adverse events observed, 62 gilts were inoculated on July 20, and the remaining 5 gilts were kept as controls. The gilts were inseminated 5- or 9-weeks post inoculation. Using quantitative PCR (qPCR), it was estimated that each gilt received a dose of approximately 1500 genomic copies of PK.

No clinical signs were noted following inoculation. Paired sera from 10 inoculated gilts and from the 5 control gilts were evaluated for PK titers using an enzyme-linked immunosorbent assay (ELISA) under development at Iowa State University (Figure 1). Four of the five control gilts had virtually no antibodies at the first sampling. Three of the control gilts remained negative and the fourth gilt strongly seroconverted. One control gilt initially had a relatively high titer and remained about the same through the second sampling. Of the 10 inoculated gilts, 3 had almost no antibodies initially but did seroconvert. The 4 inoculated gilts with intermediate titers saw their titers decline by the second sampling, and the 3 gilts with high titers at the first sampling had approximately the same titer levels at the second sampling.

Table 1: Incidence of CT and SL piglets from 259 litters farrowed 7 months (June 2020) after observation of the first cases

| | Litters, No. | Piglets with CT | | Piglets with SL | |
		Total	Mean	Total	Mean
Unaffected	204	0	0	0	0
CT only	8	48	6.0	NA	NA
SL only	32	NA	NA	51	1.59
Both	15	88	5.87	64	4.27
Total	259	136	NA	115	NA

CT = congenital tremors; SL = splayleg; NA = not applicable.

Table 2: Preweaning mortality associated with splayleg during the first and last 7 batches of weaned pigs

| Batches | Weaning dates | Total piglets born live | Piglets born live/litter, mean | Total preweaning mortality, % | Splayleg | |
					Mortality, %	% of total mortality
First 7	Dec 2019 - Jun 2020	23,157	12.74	17.87	5.39	30.16
Last 7	Mar 2021 - Aug 2021	24,144	13.99	18.18	1.06	5.83

Figure 1: Paired serological titers of 5 control gilts and 10 gilts inoculated with sera from pestivirus K-positive piglets.
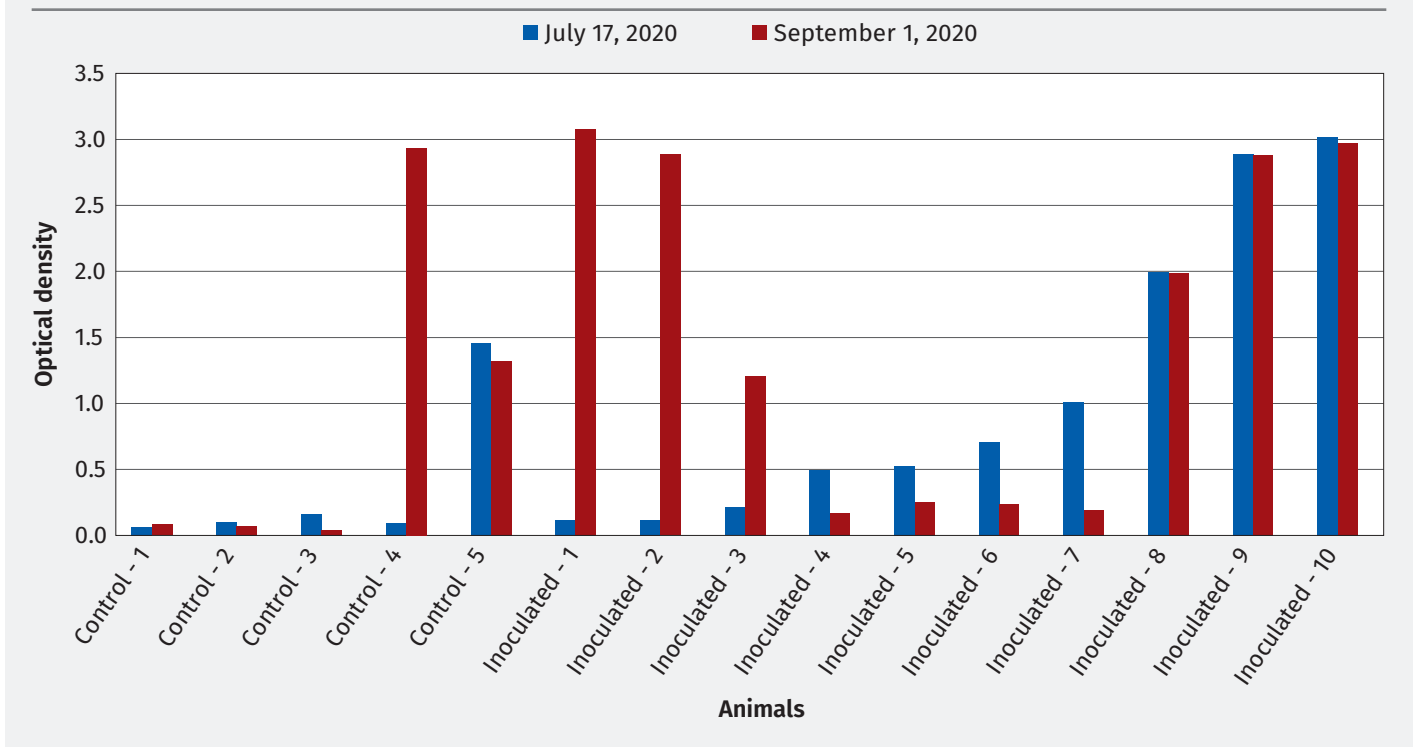
The first batch of inoculated gilts (31) farrowed in late December 2020. At that time, the losses associated with CT and SL were becoming minimal, which made it more difficult to determine if the inoculation strategy had an impact or not. One gilt had 5 piglets with CT, while 5 gilts had a total of 7 piglets with SL.

Discussion

A clear association between CT and PK has been made in previous studies.[3-5] However, the association between SL and this virus is not as clear. Different causes or factors have been proposed to explain the occurrence of SL including slippery floors, large litters, low birth weight, choline or methionine deficiency, mycotoxins, genetics, short gestation lengths, and inducing farrowing too early.[5] Madsen et al[5] did identify CT as a condition to which SL can be associated without specifying if PK could be considered as a causal agent. In this case, as seen in Table 1, more litters (32) had only SL piglets compared to only CT piglets (8). Thus, the incidence of SL was not conditional to the presence of CT in a litter. Nevertheless, litters with both problems had a higher mean number of SL piglets (4.27) than litters with only SL piglets (1.59), so there appeared to be a predisposition to SL in litters with CT piglets.

The role PK played in the occurrence of SL in the case herd cannot be confirmed. But, there is increasing evidence that the virus, while not the sole cause, may be associated with this condition. When inoculating sows with PK on day 45 or 62 of gestation, Arruda et al[3] reported that 75% and 17.5% of the piglets were affected with CT and SL, respectively. In one litter, all piglets (10 of 10) had CT and 4 of them also were splaylegged. In another study where 3 gilts were experimentally infected with the virus on day 32 of gestation, 2 of them produced piglets with CT (11 of 13 and 13 of 15) and SL (3 of 13 and 7 of 15), with some piglets affected with both conditions.[4] Under field conditions, Sutton et al[6] described a case on a high-health research farm in the United States where the prevalence of SL was 33% in pigs with CT, and 0.8% in unaffected pigs. All tested litters with CT (41) had pigs positive for PK by qPCR, while the litters without CT (50) had no PK-positive pigs. Similarly on two Brazilian farms with an abrupt increase of CT, 29.7% (102 of 343) and 44.2% (19 of 43) of the piglets with this condition also had SL. Pestivirus K was identified by PCR in all 13 piglets with CT that were tested and in 1 of 6 unaffected piglets.[7] Schwarz et al[8] reported that a fatal combination of CT and SL was observed in an Austrian herd, but in single piglets. When other herds

with CT were investigated, 3 of 5 herds reported concomitant problems with SL. Finally, White[9] stated that it was common for CT pigs to also show SL.

The chronological association between CT and SL problems in field situations, coupled with the experimental reproduction of both CT and SL in gilts inoculated with PK during pregnancy, seems to leave little doubt as to a possible association between PK and SL. This is not to say that PK will necessarily produce SL pigs or that other causes or factors cannot be associated with it. Two of five herds investigated in the Schwarz et al[8] study did not report concomitant SL issues. In 4 Swedish farms in 2017-2018, 13 piglets with SL were tested and all were found to be PK-negative by PCR.[10] In Denmark, no reports of concomitant SL were mentioned in 10 herds with CT problems where all affected piglets tested (55) were found to be PK positive.[11]

What is currently known seems to suggest that there are situations where PK infection may be associated with SL problems, but not necessarily in others. Differences between PK strains have been identified.[12,13] It could be that some strains may be more likely to be associated with SL than others. In the case herd described here, the mean number of SL piglets in litters that also had CT

piglets was 2.69 times higher (4.27 vs 1.59) than in litters with only SL piglets. This suggests that litters in that herd with CT were more likely to also have SL problems.

The reason why the case farm broke with these 2 conditions and why it lasted so long is unknown. The current understanding is that for CT type A-II, nonimmune females that come in contact with the virus at a certain time in gestation may produce affected piglets. After infection, long-term immunity seems to develop as it appears rare for the same female to produce more than one affected litter.[4] In herds that have been established for a while, the condition can affect litters of different parities, but is more often seen in gilts.[4,7,9] New herds are particularly at risk in terms of losses that can be associated with CT.[9]

Seven months after the first clinical signs were observed in the case herd, 52 of the 55 litters affected with CT, SL, or both were from parity 2 sows. It is hypothesized that these females had not come in contact with the virus before their second gestation, and that they had not produced an affected litter during their first parity. Changes in farm personnel resulted in difficulties to compile accurate data. While uncommon, long-term problems with CT have been reported where the condition was present for several months and sometimes more than a year.[4,6]

It is believed that most herds are likely infected with PK. In a collection of sera from multiple US states, 94% of samples were found to be seropositive for PK using an ELISA. Further sampling from 3 farms revealed that 2 farms had 96% and 100% seropositive sera, while the third farm had none.[1] Consequently, introduction of PK-naive gilts into infected herds is a possibility that needs to be considered. Similarly, the virus has been detected by PCR in semen coming from different commercial US boar studs, and the role this could play in the epidemiology of the infection needs to be assessed.[14]

The case farm was populated from 5 different gilt developer units filled with gilts from 6 sow herds, but the source of the gilts could not be identified once introduced into the sow herd. Thus, it is plausible that gilts coming from one or more of these gilt developers had not come in contact with PK before their introduction into the sow herd being populated. This is supported by the serological data (Figure 1). Of the 15 tested gilts, 7 had few or no antibodies at the first sampling. All 3 inoculated gilts with initially few or no antibodies showed a strong increase at the second sample, suggesting that these animals had not been exposed to the virus before being introduced into the newly populated herd. Conversely, the 3 inoculated gilts with high titers at the first sampling basically maintained the same titer levels after inoculation. Ideally, efforts should be made so that gilts come from only one source, but in cases where it is not possible, mixing the gilts from different sources early before their introduction into the sow herd would seem to increase their chances of coming in contact with the virus and becoming immune before their first gestation. White[9] suggested that placing gilts in contact with 8- to 12-week-old pigs for 4 weeks and ending at least 2 weeks before service appeared to provide satisfactory exposure.

Following initial cases of CT, Sutton et al[6] orally exposed 91 gilts to an inoculate obtained from fetal fluids and membranes collected from sows that had produced CT-affected litters. This was done 54 days prior to insemination with the goal to immunize the gilts before they became pregnant. Yet 45.0% of the litters produced and 30.8% of all piglets were affected by CT. Thirty- three percent of the piglets affected with CT had SL, compared to 0.8% in unaffected piglets. The inoculation strategy used in the herd described in this case report did not seem to have a significant impact on the condition and losses. The clinical situation had already vastly improved when inoculated gilts farrowed, which made interpretation difficult. Still, 1 inoculated gilt produced 5 piglets with CT, and there was no difference between the number of SL piglets from the gilts administered the presumably infected serum and in the two batches that preceded the inoculated batch. It is also perplexing to see that 52 of the 55 females with affected litters in July 2020 (7 months after the first cases were observed) were of parity 2 and had been in the herd for about a year. This should have been enough time for gilts to become infected and immune before producing affected litters. More work is needed to identify procedures that can be applied to effectively prevent these conditions, particularly for new herds that need to use more than one gilt source. Given the differences between PK strains, one area that needs clarification and that can have an impact on control measures is the level of cross protection that is obtained against different strains following infection with a single strain.[12,13,15]

Serologic assays have been developed, but their usage is recent. Once more is known about what is to be expected under field conditions from these assays, they could become useful tools to determine if interventions are needed or not. In the case described here, the serological results obtained following the inoculation protocol are difficult to interpret and do not allow for conclusions to be made on its efficacy. The 3 inoculated gilts with very low initial titers did strongly seroconvert, but so did 1 control gilt that did not receive the serum from infected piglets. Whether the seroconversion was associated with virus shed by the inoculated gilts, or by contact with already infected animals is unknown. It is possible that the serum used to inoculate the gilts was not infectious and did not influence the results obtained. A few inoculated animals had lower titers at their second sampling, a situation that can be observed in animals with declining maternal immunity. Limited information is known about the duration of maternal immunity to PK. In 2 studies where this was investigated, it varied between 3 and 8 weeks which would seem to eliminate the possibility for declining maternal immunity to be involved in the current case given that the gilts were approximately 26 weeks of age at the time of inoculation.[8,16] The declining titers could also be a reflection of animals that had been exposed to the organism in the past and were towards the end of the detectable antibody duration. In one study that evaluated the duration of antibodies in a CT-affected herd using an ELISA, healthy piglets from a healthy litter were positive after birth and became negative at 3 to 6 weeks of age when maternal immunity waned. Following infection, the piglets were positive again at 70 days of age and were still strongly positive at 160 days of age. In that case, duration of actively acquired antibodies lasted at least 3 months.[16] The assay used in the current case report was under development at the time and had not yet been fully validated. Thus, more work is needed before the strengths and limitations of the assay are determined.

A few weaknesses of this case report are readily acknowledged. First, the number of pigs with both conditions and the associated mortality should have been

compiled. The litter mortality records used by the personnel included SL as a cause, but not CT. Second, necropsy of a few SL pigs could have helped to clarify the role of PK in that condition. The simultaneous appearance of both SL and CT suggested a common cause, and it was initially felt that the problems would be temporary and not persist as long as they did. Thus, there was no plan at the time to report the findings. The duration of the conditions and their significance, particularly that associated with SL, later suggested that reporting what was observed could be of value.

Finally, losses associated with CT can be significant. In a small new herd of 400 sows, it was estimated that 1000 piglets were lost.[9] Schwarz et al[8] reported that in 5 Austrian herds affected with CT, the losses went from almost none to the equivalent of 4.9 to 7.3 pigs/sow/year. In the case described here, the number of piglets that died because of CT could not be quantified but was estimated to be low. However, the losses associated with SL alone were estimated at more than 1000 pigs.

Implications

Under the conditions of this case report:

- Pestivirus K may be associated with both CT and SL.
- Losses associated with PK can be significant and last for several months.
- More work is needed to identify preventive methods, particularly for new herds.

Acknowledgments

Conflicts of interest

None reported

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