

SHIC Diagnostic Investigation: Tremors/pasivirus/unknown (draft)

Abstract

In late spring/early summer of 2015, a grow-finisher flow in the Southeast US experienced increased death loss associated with acute onset of CNS signs initially described as tremors. From one submission a diagnosis of streptococcal septicemia exacerbated by metabolic bone disease was offered as presumptive diagnosis. Another submission resulted in no lesions of diagnostic significance being detected in multiple tissues, including brains, lungs, livers, kidneys, spleens, lymphoid tissues, intestines, stomachs, hearts or skeletal muscles. And no etiologic diagnosis was identified nor were any microscopic lesions suggestive for infectious insult to the CNS observed.

Additional tissue and serum samples from two groups of clinically affected pigs were submitted for next-generation sequencing (NGS). A novel pestivirus was identified from the brain samples from this herd by NGS. No other viruses were detected in the brain tissue.

The pestivirus detected by NGS was similar to that reported in pigs born with congenital tremors caused by a novel pestivirus, and similar to a pestivirus previously named "atypical porcine pestivirus" or APPV. The presence of APPV in the brain was further confirmed by qRT-PCR. APPV was also identified in other tissues (lymph nodes, spleen, liver and lungs) by qRT-PCR and immunohistochemistry. Although APPV was found in samples from affected pigs in this case, proof as actual cause of this clinical disease remained lacking.

This grow-finish flow continued to have sporadic cases of this clinical malady. For this reason, with Swine Health Information Center support, three pigs with typical early signs of tremors and 2 normal cohort pen mates were euthanized and examined. Blood was collected from shaker pigs prior to transport to a necropsy facility. Also collected and retained were oral fluids and "convalescent" serum from pen mates. Additional history and clinical signs were reviewed.

Necropsy and pathologic examinations of the 5 pigs (3 affected and 2 normal cohorts) reportedly revealed only very mild bronchopneumonia (<5% cranioventral lung consolidation) as the only gross lesion observed in any of the pigs. Examined tissues included fresh and fixed brain, spinal cord, tonsil, lung, thymus, heart, liver, spleen, kidney, adrenal, skeletal muscle, stomach, intestine, colon blood and serum. Lesions of diagnostic significance as related to clinical presentation were not detected in any tissue from each of 5 pigs.

In addition, a PCR for PCV2 was negative in the affected group but positive, with Ct of 21, in a pooled sample from unaffected cohorts. PCR for PRRSV was scanty positive in lung but negative in brain tissue. Two cooperating laboratories confirmed PCR for congenital tremors/pestivirus/APPV was negative on each of 5 brains. Next generation sequencing (NGS) on pooled brain tissues did not have a predominating genome detected. However, NGS of serum demonstrated fairly high levels of swine pasivirus (a member of the *Picornaviridae* family) as well as lesser amounts of parvovirus type 7. It is not known what, if any, pathological effect these viruses may have. Transient and subclinical viremia is common with many viral infections, including nonpathogens.

A definitive diagnosis for cause for this unusual and severe fatal tremor disease was not offered by this investigation. Abundant pasivirus was detected by NGS in serum samples but not neural tissue. This result was considered unusual and has not been a common finding. Interestingly, pasivirus was originally discovered/reported a few years ago from healthy pigs, but there is a recent report from Europe where this virus was found in paraplegic pigs by sequencing.

It should be emphasized that many viruses are endemic in swine populations and merely detecting a novel virus with molecular techniques in a sample is not definitive evidence for disease causation. Unfortunately, proper investigation tools are not in place to elucidate pathogenicity, virulence, prevalence for most of these types of newly discovered viruses, including pasivirus. Serology tests have not been developed and animal studies have not been performed. Additional research is needed to determine if pasivirus may have played a role in this case.

Herd History

In late spring/early summer of 2015, a grow-finisher flow in the Southeast US experienced increased death loss associated with acute onset of CNS signs initially described as tremors. Early laboratory submissions from this flow did not include brain or nervous tissue; endemic bacterial pathogens were detected from submitted tissues.

A complete set of tissues, including brain, was submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISUVDL) in April of 2015 from 7- to 8-week-old pigs weaned 3 to 4 weeks. Tissues were from pigs with muscle fasciculation and tremors, but animals were still ambulatory with central awareness. Clinical signs progressed to paresis, sternal, then lateral recumbancy. Most affected pigs died within 3 days but a few sudden deaths were also reported. Affected pigs were scattered throughout the barn. Rigor reportedly occurred rapidly after euthanasia. Rib bones were reported to bend before breaking. Laboratory testing was conducted to rule out differentials of PRRSV, enterovirus, hemolytic *E. coli*, bacterial meningitis, and nutrient (e.g. vitamin D, calcium, phosphorus, vitamin E and selenium) deficiency. Tissues from each of four pigs were submitted and found to have suppurative meningitis and fibrinopurulent polyserositis associated with the presence of *Streptococcus suis* in brains from all 4 pigs as well as active PRRSV infection. High populations of hemolytic *E. coli* were isolated from intestines but adherent bacteria to enterocytes were not detected by microscopy. Vitamin D levels were below normal (i.e. 50% of normal values) and bone ash was below normal limits as well. No microscopic evidence for other disease processes was reported. A diagnosis of streptococcal septicemia exacerbated by metabolic bone disease was offered as presumptive diagnosis.

In May of 2015, another complete tissue submission was received by ISUVDL from 14-week-old pigs placed 4 weeks in the finisher. These were from the same flow as the previous case and had very similar clinical signs reported. A complete set of tissues from 3 pigs was examined with no lesions of diagnostic significance detected in multiple tissues, including brains, lungs, livers, kidneys, spleens, lymphoid tissues, intestines, stomachs, hearts or skeletal muscles. No etiologic diagnosis was identified nor were any microscopic lesions suggestive for infectious insult to CNS observed. Differentials suggested included metabolic disease, nutritional disease, genetic abnormality or insult from toxin, examples of which might include: cerebellar abiotrophy, thiamine responsive encephalopathy, tremorgenic toxins or metabolic bone disease. It was suggested there be serum chemistry performed on acutely affected pigs for Ca, P, Mg and vitamin D as well as a general large animal panel and urinalysis.

Around this time (early summer of 2015) tissue and serum samples from two groups of clinically affected pigs were submitted by attending veterinarians to researchers at Kansas State University Veterinary Diagnostic Laboratory (KSUVDL) for analysis, specifically next-generation sequencing (NGS). A novel pestivirus was identified from the brain samples from this herd by NGS. No other viruses were detected in the brain tissue. Submitters at this time reported a history of uncontrollable shaking and intention tremors which resulted in the death of all pigs affected. The pestivirus detected by NGS was similar to that reported in pigs born with congenital tremors caused by a novel pestivirus, and similar to a pestivirus previously identified by the KSUVDL group who named it "atypical porcine pestivirus" or APPV. This virus was reported as a novel, highly divergent pestivirus in swine and suspected to be widely distributed in the U.S. swine herd. The presence of APPV in the brain was further confirmed by qRT-PCR. APPV was also identified in other tissues (lymph nodes, spleen, liver and lungs) by qRT-PCR and immunohistochemistry. Although APPV was found in samples from affected pigs in this case, proof as actual cause of this clinical disease remained lacking. In the meantime, this grow-finish flow continued to have sporadic cases of this clinical malady. For this reason, SHIC was contacted by herd the veterinarians for assistance in further diagnostic investigation.

SHIC Investigation

With SHIC support, three pigs with typical early signs of tremors and 2 normal cohort pen mates were euthanized and examined. Blood was collected from shaker pigs prior to transport to a necropsy facility. The submitting veterinarian noted that affected pigs "over-reacted" to the snare, as if hypersensitive to restraint in that they fought with vigorous thrashing in contrast to routine and expected behavior in snared cohorts. Also collected and retained were oral fluids and "convalescent" serum from pen mates. Additional history and clinical signs were reviewed, including:

- Pigs first started showing unusual nervous system clinical signs in April of 2015, evidenced by submissions described above.

- Samples in this SHIC-assisted submission were from pigs approximately 14 weeks of age, about 4-5 weeks on feed in the finisher. The onset of clinical signs started late in nursery but new cases were reported to persist all the way through the growing period nearly to marketing.
- Clinical signs observed and described by submitter as starting as a mild full-body tremor. In the earliest stages pigs are able to walk, eat, and drink; however, there is increasing progression of severity to the point where tremors are sufficiently severe to compromise ambulation, eating, and drinking and therefore affected pigs must be euthanized. Interestingly, pigs do remain centrally alert and cognizant of their surroundings in contrast to most CNS cases that have frank meningitis or encephalitis. The clinical progression from the onset to severe clinical signs is about 3-4 days at which time they are humanely euthanized. If an affected pig is not removed from general population as soon as clinical signs appear, the other pigs will aggressively savage it and often pigs will be found dead.
- This condition appears to have started with the weaned pigs immediately after the sow farms broke with PED, leading one to consider either simultaneous introduction of another virus or perhaps some compromise in nutrient absorption or metabolism.
- There are multiple groups affected, but no new cases were reported in the nursery phase at the time of submission (the nursery signs have stopped). The oldest pigs affected have been on 3 different feed rations and new cases continue to occur.
- During the period of investigation, this unusual neurologic syndrome appeared at multiple nursery and finishing sites in this flow, all with different management and separated by several miles.

Necropsy and pathologic examinations of the 5 pigs (3 affected and 2 normal cohorts) reportedly revealed only very mild bronchopneumonia (<5% cranioventral lung consolidation) as the only gross lesion observed in any of the pigs. A complete selection of well-preserved tissues was collected from each individual pig at the necropsy site and submitted to ISUVDL for laboratory investigation and histopathology. Examined tissues included fresh and fixed brain, spinal cord, tonsil, lung, thymus, heart, liver, spleen, kidney, adrenal, skeletal muscle, stomach, intestine, colon blood and serum. Lesions of diagnostic significance as related to clinical presentation were not detected in any tissue from each of 5 pigs.

Additional laboratory testing included:

- PCR for PCV2 negative in group affected but positive with Ct of 21 in pool of unaffected cohorts
- PCR for PRRSV scanty positive in lung but negative in brain tissue
- PCR for congenital tremors / pestivirus / APPV was negative on each of 5 brains, both at ISUVDL and KSUVDL
- Blood chemistry demonstrated hyperkalemia to be present in affected pigs; however, hemolysis can falsely elevate potassium. There was considerable anion gap in affected pigs, the cause of which is unknown.
- Next generation sequencing (NGS) was performed by KSUVDL. Brain pools did not have a predominating genome detected. However NGS of serum demonstrated fairly high levels of swine pasivirus (a member of the *Picorniviridae* family) as well as lesser amounts of parvovirus type 7 but it is not known what, if any, pathological effect these viruses may have. Transient and subclinical viremia is common with many viral infections, including nonpathogens.

Discussion

A definitive diagnosis for cause for this unusual and severe fatal tremor disease was not offered by this investigation. Next generation sequencing found APPV in initial submissions to KSUVDL but this virus was not detected in other submissions or subsequent submissions. Evidence for enteroviruses, sapelovirus or a host of other swine viruses associated with CNS disease in pigs was not detected by this investigation, nor is there definitive evidence that this condition is infectious or transmissible. Transmission studies (i.e. inoculation of susceptible pigs with crude inocula) perhaps could confirm transmissibility. Alternative causes, including toxins, nutritional derangement, metabolic disease or other anomalies are neither implicated nor ruled out.

Abundant pasivirus was detected by NGS in serum samples but not neural tissue. This result was considered unusual and has not been a common finding at the KSUVDL. Other members of the family *Picornaviridae*, to which pasivirus belongs, are many, known to infect mammals and in some cases,

cause disease. Interestingly, pasivirus was originally discovered/reported a few years ago from healthy pigs, but there is a recent report from Europe where this virus was found in paraplegic pigs by sequencing (Boros et al, 2016, Arch. Virol. 160: 1363-6). Related human pasiviruses have been associated with flaccid paralysis (Williams et al., 2009, J. Gen. Virol. 90:1702-12). Assembly of the complete genome of this particular pasivirus by KSUVDL researchers revealed 83% and 90% identity to closest pasivirus reported in literature, suggesting this virus may be novel. It should be emphasized that many viruses are endemic in swine populations and merely detecting a novel virus with molecular techniques in a sample is not definitive evidence for disease causation. Unfortunately, proper investigation tools are not in place to elucidate pathogenicity, virulence, prevalence for most of these types of newly discovered viruses, including pasivirus. Serology tests have not been developed and animal studies have not been performed. For example, in this case further testing (e. g. in situ hybridization) could be used to detect pasivirus in neural tissue as a first step in establishing causality for pasivirus.

Thiamine-responsive polioencephalomalacia has been described in pigs; however submitting veterinarian reported no benefit from supplemental thiamine injections. Several abnormalities in clinical pathology are noteworthy but not definitive for a particular etiology including:

- Hyperkalemia may be artifact or perhaps could be related to metabolic disease (myotonia / myoclonia) similar but not identical to that reported in periodic hyperkalemia in horses.
- Hyperphosphatemia was consistently present as well. Renal disease and vitamin D toxicity are sometimes associated with hyperphosphatemia; however, lesions in kidney and ectopic mineralization are not features in this case.
- Acute hypocalcemia is not present, suggesting metabolic bone disease is not likely to be consistently involved.
- An increase in anionic gap is present and can occur with exposure to organic acids (salicylates, alcohols, ethylene glycol, methanol, paraldehyde, toluene, etc.) and, in humans, there is also a metabolic derangement of pyroglutamic acid described. Speculation could include a role for additives (e.g. formaldehyde, propionic acid, organic acids, mycotoxin inhibitors or binders) in feed. Another source, aspirin administration, was not associated with outbreaks.

The morbidity and mortality of this unique clinical disease within this production flow was variable but occurred from roughly March of 2015 until March of 2016. Presently, no new cases have been observed in nursery phase since March 2016, and all finishing lots with reported clinical signs have been closed out. No new cases have been reported in the past 6 months in this flow. No transfer of biological materials has been allowed between the three sister farms supplying pigs to the wean-finish flow.