RESEARCH REPORT

SWINE HEALTH

Title: Porcine circoviral associated disease: Lesions, concurrent infectious agents, efficacy of diagnostic tests in clinical samples and tissues and comparison of genomic homology. – **NPB #98-230**

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I. Abstract:

Thirty-four pigs, 2 each from 17 farms with clinical PMWS, were selected for study. No management practices common to all farms were discovered as potential predispositions to PMWS. PCV2 was demonstrated in all 34 pigs in association with lesions previously described for PMWS. In addition, enzootic pneumonia was confirmed in 24 pigs, PRRS in 2 pigs and septicemic salmonellosis in 2 pigs. The most consistent tissues with microscopic lesions suggestive of PMWS were lungs (100% of pigs), livers (88%), ileums (82%), enlarged lymph nodes (82%), stomachs (79%) and kidneys (68%). The prevalence of lesions in other tissues ranged from 50 to 6%. Viral inclusion bodies typical of PCV2 were observed in only 21% of PCV2-positive pigs. PCR was the most sensitive test to detect PCV2 (65% of tissues were positive), followed by in-situ hybridization (43% were positive) and virus isolation (22% were positive). The tissues from which PCV2 was most consistently demonstrated included enlarged lymph nodes (100% of pigs), ileum (100%), spleen (88%) and tonsil (68%). Lesions typical of PMWS were most commonly demonstrated concurrently with PCV2 in enlarged lymph nodes (82% of pigs) and ileum (82% of pigs), suggesting that these tissues would be the most useful for diagnostic testing. Virus isolation, PCR and IFA tests for other viruses identified porcine adenovirus in 74% of pigs and PRRS virus in 6% of pigs. No pigs tested positive for porcine parvovirus, pseudorabies virus, swine influenza virus, TGE virus or rotavirus. Twelve of the 34 pigs originated from 7 farms where the primary observed clinical manifestation of PMWS was wasting disease in association with diarrhea. Although no other infectious causes of diarrhea were demonstrated in affected pigs, lesions typical of PMWS were no more severe in the digestive tract of pigs with diarrhea compared to those with normal stools.

The entire genomes were cloned and sequenced from 4 isolates of PCV2 from 4 of the 17 farms in this study, 2 recent PCV2 isolates from neonatal pigs from 2 farms with congenital tremors (CT) and 1 isolate of an unknown type of PCV obtained in the late 1960's from a pig with CT. The 4 PMWS-PCV2s shared 99% nucleotide (nt) sequence identity with each other, and over 96% with all previously reported sequences of PMWS-PCV2s. The 2 recent CT-PCV2s shared 99% nt sequence identity with each other and also with our PMWS-PCV2 isolates. There were no consistent genomic

differences between our recent PMWS and CT PCV2 isolates. The old CT-PCV shared 98% nt sequence identity with PK-15 PCV1 and only 72% with our new CT-PCV2s, indicating that it is the first sequenced field strain of PCV1 and the first PCV1 associated with disease.

II. Introduction:

Porcine circovirus (PCV) was first isolated as a noncytopathic contaminant of a porcine kidney cell line (PK-15)[29] and has been characterized as a small icosahedral DNA virus.[27] The viral genome has been sequenced [17] and the virion has been characterized ultrastructurally.[24] Inoculation studies in pigs using PK-15 derived PCV have not resulted in clinical disease,[2, 28] Field isolates of PCV have been associated with a recently described postweaning multisystemic wasting syndrome (PMWS) in pigs[1, 3, 5, 6,7] and with CT type A2.[9, 12, 13]

Isolates of PMWS–associated PCV are genetically and antigenically different from the PK-15 cell PCV.[3, 10, 17, 19] Isolates of PCV that are genetically like PK-15 cell PCV are referred to as PCV1 and those like the first characterized PMWS isolates as PCV2.[17] Only PCV2 has been associated with PMWS. The genetic types of PCV associated with CT are not described.

Postweaning multisystemic wasting syndrome has been diagnosed with increasing frequency in the U.S., Canada and Europe.[3, 7, 11, 14, 16, 19, 22] The syndrome is characterized by progressive weight loss, dyspnea, tachypnea and icterus in postweaned pigs of approximately 8-12 weeks of age.[5] Gross lesions in pigs with PMWS consist of generalized lymphadenopathy in combination with one or more of the following lesions: (1) cutaneous pallor and icterus, (2) interstitial pneumonia, (3) hepatitis, (4) grossly enlarged, edematous kidneys with a semi translucent appearance, (5) an enlarged, meaty, noncongested spleen, (6) fluid-filled, thin-walled sections of lower intestine, with occasional edema of the wall of the cecum, and (7) gastric ulcers and gastric wall edema.[7, 14] The most distinctive microscopic lesions in affected pigs are lymphoid cell depletion and granulomatous inflammation in lymphoid organs with inconsistently occurring clusters of intensely basophilic intracytoplasmic inclusion bodies in macrophages.[7, 11, 14, 18] Other lesions include interstitial lympho-histiocytic pneumonia, lympho-histiocytic periportal hepatitis with occasional hepatocellular necrosis, lympho-histiocytic and eosinophilic peripelvic nephritis with nonsuppurative perivasculitis, lympho-histiocytic infiltration of gastric, cecal, and colonic mucosa and marked edema of the submucosa, and lympho-histiocytic interstitial pancreatitis.[7, 18] Porcine circovirus has been consistently demonstrated in large amounts within lesions and is implicated as the most likely primary cause of PMWS.

Inoculation studies in pigs with PCV2 alone have not reproduced severe clinical PMWS. Little clinical disease and mild lesions of PMWS were produced in PCV2-inoculated cesarean-derived-colostrum-deprived (CDCD) pigs.[1] More recently mild clinical disease and severe lesions compatible with those of PMWS have been reproduced in gnotobiotic pigs experimentally inoculated with PCV isolated from a pig with naturally occurring PMWS.[24] Moderate to severe wasting disease with typical lesions has been reproduced in caesarean derived/colostrum deprived pigs and in germ-free pigs infected dually with PCV2 and porcine parvovirus (PPV)[1,8] and in gnotobiotic pigs inoculated with a cell-free filtered tissue homogenate containing PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV).[24] The pathogenesis of PMWS remains unclear, but it seems that PCV2 is primarily the cause of PMWS yet that it requires augmentation by a second virus. It is unknown whether PPV and PRRSV

are the only viruses that can augment PCV2 and it is unknown which viruses most are most commonly involved in field cases of PMWS.

The role of PCV in CT type A2 is unclear. Studies that suggest a role for PCV were done prior to the discovery of PCV2, thus the type of PCV used in these historic studies remains unknown. Inoculation of pregnant sows during gestation with a virus morphologically like PCV reproduced congenital tremors (CT).[9, 12, 13] In one of these studies, the virus was purified and identified as PCV by morphology and indirect immunologic methods.[12] However, the genetic type of the circo-like virus was not determined and the virus was lost to further study. In other of these studies, the circo-like virus was saved and is available to our research group for further study.[9, 13] Recently, PCV2 was detected in large numbers of neurons in the brains and spinal cords of neonatal pigs with CT.[25] It is not known whether these neuron-associated PCV2 would cause CT if inoculated into naïve pregnant swine. Although complete genomic sequences of a number of PMWS PCV2 are available, genetic analysis of PCV isolates associated with CT is not yet reported.

Diagnosis of PMWS relies on the recognition of characteristic gross and microscopic lesions associated with characteristic viral inclusions in the cytoplasm of infected macrophages.[5] Infection with PCV2 can be confirmed with immunohistochemistry, in-situ hybridization or PCR.[6, 7, 19] However, for general diagnostic use, the relative sensitivity of various tests and the best tissues for testing, are not known.

IV. Project Objectives

1. To determine correlations between herd clinical history, severity and distribution of gross and microscopic lesions, PCV distribution in tissues and concurrent infectious agents.
2. To determine the best test to detect virus i.e. PCR, in-situ hybridization, virus isolation or immunohistochemistry.
3. To determine the optimal tissues from which to confirm viral infection.
4. To compare the sequences of historic (isolated 25-30 years ago in Indiana) contemporary isolates of PCV isolated from pigs with PMWS and CT.

V. Procedures:

Study Design – From Indiana farms previously confirmed with PMWS, 2 typical clinically affected pigs per herd were selected for testing. Farm health history and management practices were determined by a survey. Complete necropsy examinations were performed and gross lesions were recorded. Samples were collected for histopathology and other testing. Tests for PCV included virus isolation (VI), polymerase chain reaction (PCR) and in-situ hybridization (ISH). Tests for other viruses included VI and rtPCR for PRRSV, PCR for PPV, PCR for porcine adenovirus (PAV), direct fluorescent antibody (FAT) for transmissible gastroenteritis virus (TGEV), rotavirus group A (RVA) and swine influenza virus (SIV) and VI on swine kidney and swine testicular cells for other cytopathic viruses. Tests for bacteria included culture and PCR for *Salmonella sp., Brachyspira (Serupulina) hyodysenteriae* and *pilosicoli*, PCR for *Lawsonia intracellularis* and culture for *E. coli*, *Hemophilus parasuis* and *Streptococcus suis.* Seven PCV isolates were selected and the entire genomes were cloned, sequenced and compared with each other and previously reported PCV sequences. Four isolates (PMWS-PCV-P1, P2, P3 and P4) originated from 4 pigs with PMWS from 4 different farms in this study. Each isolate had a different pattern of amplification with 4

sets of primers in PCR, suggesting genomic differences. Two isolates (CT-PCV-P5 and P6) were recently obtained from 2 neonatal pigs with CT from 2 different farms.[25] The last PCV (CT-PCV-P7) was isolated in primary central nervous system (PCNS) cells in the late 1960's by Kanitz from a neonatal pig with CT.[13]

Samples from Pigs with PMWS selected for Testing – Tissue samples for histopathology and ISH included mesenteric, bronchial and inguinal lymph nodes, spleen, thymus, tonsil, bone marrow, lung, liver, kidney, pancreas, stomach, ileum, cecum and colon. Tissue samples for VI and PCR were the same as for histopathology excepting pancreas, cecum and colon were not included. Serum, lung and spleen were tested for PRRSV and only lung for SIV. Lung, kidney, mesenteric lymph node and ileum were tested for PAV. Ileum and jejunum were tested for TGE and RVA. Ileum, colon and mesenteric lymph nodes were tested for *Salmonella sp*. Colon and cecum were tested for *Brachyspira sp.* Ileum and colon were tested for *Lawsonia intracellularis*. Lung, spleen and liver were cultured for aerobic bacteria and cytopathic viruses.

In-situ hybridization – ISH was performed using a PCV specific oligoprobe. Briefly, tissue sections from sows, uterine and day 0 control pigs, PCV contact control pigs, PCV and TH inoculated pigs were deparaffinized, proteolytic digested with 0.25% pepsin and then prehybridized. Hybridization was performed for 5 minutes at 105^0C and 60 minutes at 37^0C with a specific 3'-end digoxigenin labeled oligoprobe at a concentration of 5µl/1ml using Fischer's microprobe workstation. The detection system consisted of the anti-digoxigenin antibody conjugated with alkaline phosphatase (dilution 1:500) and the substrates "NBT/X-Phos"(Nitro-blue tetrazolium/5-Bromo-4-chloro-3-indolylphosphate). Controls included dot-blot slides of PCV infected PK-15 cells,[26] lymphoid tissue from PCV infected pigs and sections of lung, lymph nodes and liver from PCV negative pigs.[24]

Polymerase chain reaction – For PCV, tissues were homogenized in equal volumes of MEM using a tissumizer. Total cellular DNA was extracted using a standard protocol.[21] A set of PCV1 or PCV2 specific primers were used to amplify PCV sequences by PCR using Vent DNA polymerase. For detection of PPV, one set of specific primers were designed in the genomic region homologous among different strains of PPV.[4, 20, 30] PCR amplified DNA samples were analyzed on 1% agarose gel by electrophoresis and bands were visualized by an UV transilluminator. PCR for other agents was accomplished as previously described: PPV, PAV, *Salmonella sp*., *Brachyspira sp*. and *Lawsonia intracellularis*.

Immunofluorescence – Tissues were snap frozen with solid CO_2 and 6 µm cryosections were cut, mounted on cover slips, air-dried and fixed with acetone. After washing with Tris buffer, pH 8.7, sections were incubated with fluorescein isothiocyanate (FITC)-conjugated antibodies against PRV, SIV, PHV, PHEV, PPV and TGEV at a dilution of 1:500 for 30 minutes at room temperature in a humid chamber. Any unbound stain was removed by washing with Tris buffer. Sections were counterstained with a 10^{-5} dilution of Evans blue in Tris buffer, mounted in glycerol and examined by ultraviolet light microscopy.

Virus isolation – Tissues were processed for inoculation into tissue culture by grinding them to a 10% suspension in phosphate-buffered saline (pH 7.4) containing penicillin, streptomycin, and gentamicin. The tissue homogenates were centrifuged at 3,000 rpm at 4°C for 15 min and the supernatants were filtered through a 0.45 µm syringe filter. For PRRSV isolation 0.1 ml of each filtrate or clarified undiluted serum was inoculated into each well of 24-well tissue culture cluster plate containing approximately 5 x 10^5 swine alveolar macrophages in 1 ml of minimum essential medium supplemented with 4% donor calf serum. Plates were incubated at 37^0C in a

5% CO_2 humid atmosphere. After 4-5 days, 0.2 ml of the detached cell suspensions were removed from the inoculated wells and layered onto microscope slides using a Cytospin 3 cytocentrifuge at 1,000 rpm fro 5 min. The slides were stained with porcine monoclonal PRRSV antibody (SR30-F) conjugated with FITC and examined by ultraviolet light microscopy. In addition, swine testicular cells and swine turbinate cells were grown in Leighton tubes 2-3 days followed by inoculation with 0.25 ml of filtrate or clarified undiluted serum into 4 Leighton tubes containing coverslips. Inoculated cells were observed daily for cytopathic effects. Coverslips were removed on day 2, 4, 6 and 8 and cells were stained with the panel of FITC-conjugated antibodies listed above in the section of immunofluorescence and examined by ultraviolet light microscopy. The lung samples were passed once on swine testicular cells and swine turbinate cells and restained for respiratory coronavirus using a FITC-conjugated antibody against TGEV.

Cloning and sequencing - *DNA isolation and PCR:* PCNS cells grown in EMEM were harvested when cells started floating in the medium. The cell pellet was lysed by SDS-pronase (500 µg/ml pronase in 10 mM Tris, pH7.4, 10 mM EDTA, and 0.5 % SDS) and incubated at 37°C overnight. The total cellular DNA was isolated by phenol extraction followed by ethanol precipitation. Lymph nodes for PMWS-PCV-P1, -P2, -P3, -P4, and CT-PCV-P6 and liver for CT-PCV-P5 were homogenized in EMEM using a tissumizer followed by sonication using a sonicator. Tissue homogenates were incubated with the equal volume of SDS-pronase (1 mg/ml pronase in 20 mM Tris, pH7.4, 20 mM EDTA, and 1 % SDS) at 37°C overnight. Total cellular DNA was obtained by phenol extraction and ethanol precipitation. The DNA was used as a template for PCR using Vent DNA polymerase (New England BioLab) with two pairs of primers (Table 4) to amplify the entire PCV genome. For PMWS-PCV-P1, -P2, and -P3, CT-PCV-P5, and -P6; PCV2-1 & PCV2-2 and PCV2-3 & PCV2-4 sets of primers were used. For PMWS-PCV-P4, PCV2-1 & PCV2-2 and PCV4-1 & PCV4-2 sets of primers were used. For CT-PCV-P7, PCV1-1 & PCV1-2 and PCV7-1 & PCV7-2 sets of primers were used. PCR products were analyzed on 1% agarose gel and visualized with an UV transilluminator. *Cloning of PCR products:* The PCR products were cloned into the *Sma*I site of pUC18 by blunt-end ligation using T4 DNA ligase (New England Bio Lab). To construct the entire genome of PMWS-PCV-P1, -P2, and –P3, pUC18 containing PCR products from nt 1076-679 amplified with PCV2-1 & 2 primers and PCR products from nt 7-1657 amplified with PCV2-3 & 4 primers were digested with *Stu*I and *Kpn*I. The 4 kb *Stu*I – *Kpn*I fragment from pUC18 containing PCV2-1 & 2 amplified PCR product used to insert a 1.3 kb *Stu*I – *Kpn*I fragment from pUC18 containing PCV2-3 & 4 amplified PCR product to result in pUC18 containing PCV genome from nucleotide 1076-1768 and 1-1657. The resultant plasmids containing the genome of either PMWS-PCV-P1, -P2 or –P3 were named pPCV-P1, pPCV-P2 and pPCV-P3, respectively. These plasmids when digested with *Sac*II produced the linearized-form of complete PCV genomes. Similarly PCR products obtained from other PCV strains were also cloned in at the *Sma*I site of pUC18 by blunt-end ligation. Plasmid DNA was purified by isopycnic centrifugation in cesium chloride-ethidium bromide gradients (Sambrook *et al.*, 1989). *Transfection of cloned PCV DNAs and detection of PCV:* The plasmids containing entire genome of PCV DNA (pPCV-P1, -P2, and -P3) were digested with *Sac*II to result in two fragments, the full-PCV genomic DNA and pUC18 plus a part of PCV DNA. Semi-confluent monolayers of PCV-free PK-15 cells in 6-well plates were transfected with 1µg of the ligated PCV genome using Lipofectin-mediated transfection protocol (Life Technologies, Inc.). Cells were passaged three times. After the third passage, cells were harvested, cytospined and fixed with acetone. Polyclonal antibody against PMWS-PCV raised in a rabbit (Morozov and Paul, Iowa State University, Ames, Iowa) was used for IFA. For EM, water was added to cell pellets and

the cell contents were centrifuged at 10,000 RPM for 5 min. Supernatants were collected and centrifuged at 20,000 RPM for 40 min. Pellet was resuspended in water containing 3% phosphotungstic acid and 1% bovine serum albumin. Samples were nebulized onto the carbon-coated grid and examined with a Philips 201 electronmicroscope. *DNA sequencing and sequence analysis:* Plasmids containing PCV DNA were sequenced using universal and reverse primers. Subsequently, both strands of DNA were sequenced by primer walking using an applied Biosystems 373A automated sequencer. The entire genomes of 7 PCV isolates (PMWS-PCV-P1, -P2, -P3, -P4, and CT-PCV-P5, -P6, and -P7) were analyzed using the GCG sequence analysis software (Wisconsin package).

VI. Results:

Objectives 1-3: Comparison of clinical history, lesions, PCV distribution in tissues and concurrent infectious agents in tissues of pigs with PMWS and determination of optimal tests and tissues for diagnosis of infection with PCV2 and/or PMWS.

A total of 34 pigs recruited from 17 different farms were infected with PCV2 and had lesions typical of PMWS. Affected pigs were from 6 – 20 weeks of age and ranged in weight from 30 to 148 pounds (70 # average). The clinical presentation in affected groups of pigs on all farms was of poor growth and high mortality in approximately 10-15% of pigs. In addition, the primary clinical symptoms on 6 farms were of diarrheal disease and on 11 farms were of respiratory disease. Management systems varied between affected farms. Most major types of management schemes were represented including single-site farrow-to-finish, 2 site systems that were either farrow-to-wean or farrow and nursery on site 1, and 3 site systems. Most practiced all-in/all-out pig flows in all stages. On 2 farms, multiple farrowing sites were mixed in the nursery. There was not an apparent type of management system that predisposed to PMWS. None of the farms had a recent history of congenital tremors.

Gross lesions in the 12 pigs with diarrhea included distended small and large intestines with yellow watery contents containing much indigested ground corn. The mesenteric lymph nodes were approximately 3 times normal size in 4 pigs and normal in the others. All tests for infectious causes of diarrhea were negative including TGE virus, rotavirus, *Brachyspira sp., Salmonella sp., Lawsonia intracellularis* and whipworms. Four of the 12 pigs also had firm gray consolidated bronchopneumonia suggestive of mycoplasmosis comprising 5-8% of lung parenchyma in 2 pigs and 30-40% in the other 2 pigs. All four pigs with lung lesions were positive in lungs for *Mycoplasma hyopneumoniae* and *Pasteurella multocida, Streptococcus suis* and/or *Arcanobacterium pyogenes*. Gross lesions in the 22 pigs with clinical respiratory disease were as follows. Twenty pigs had cranial ventral lobular consolidation of their lungs typical of mycoplasmosis and were positive for *Mycoplasma hyopneumoniae* and *Pasteurella multocida, Streptococcus suis* and/or *Arcanobacterium pyogenes*. Two of the pigs had multiple poorly circumscribed foci of white discoloration in the renal cortices and mudullas. The remaining 2 pigs of the 22 with clinical respiratory disease had non-collapsing rubbery lungs with cranial ventral purple consolidation from which *Salmonella cholaraesuis* was isolated. The tracheobronchial lymph nodes were enlarged 2-4 times normal in all 22 pigs as were also the ileal and mesenteric lymph nodes in 4 pigs.

Microscopic lesions were as previously described for PMWS.[5, 14] The most consistent tissues with lesions compatible with PMWS were lungs (100% of pigs), livers

(88%), ileums (82%), enlarged lymph nodes (82%), stomachs (79%) and kidneys (68%). The prevalence of lesions in other tissues ranged from 50 to 6%. The tissue previously reported as affected in PMWS in which we found the least lesions was pancreas (6% of pigs). Clusters of variably sized intracytoplasmic inclusion bodies in macrophages and giant cells typical of PCV2 viral infection were noted in only 21% of PCV2-positive pigs and were most commonly seen in ileum, enlarged lymph nodes and tonsil. In addition to lesions compatible with PMWS, lesions typical of complicated respiratory mycoplasmosis (enzootic pneumonia) were in lungs of 24 pigs and lesions typical of septicemic salmonellosis were in the lungs, livers, kidneys and lymph nodes of 2 pigs. Also, large amphophilic basophilic intranuclear inclusion bodies were identified in epithelial cells in the ileal mucosa and tonsilar crypts of 5 pigs from 3 farms. Transmission electron microscopy demonstrated intranuclear virions morphologically compatible with PAV.

All pigs were positive for PCV2 by PCR and in-situ hybridization. PCR was the most sensitive (65% of tissues were positive), followed by in-situ hybridization (43% of the same tissues were positive) and least sensitive was virus isolation (22% of the same tissues were positive). The tissues from which PCV2 was most consistently demonstrated included enlarged lymph nodes (100%), ileum (100%), spleen (88%) and tonsil (68%). Virus isolation, PCR and IFA tests for other viruses identified PAV in 74% of pigs and PRRS virus in 6% of pigs. No pigs tested positive for PPV, PRV, SIV, TGE virus or rotavirus.

The clinical respiratory disease reported in 22 of the 34 pigs studied was associated with respiratory mycoplasmosis in 20 pigs and respiratory salmonellosis in the other 2 pigs. Although all lungs had lesions suggestive of concurrent PMWS i.e. expanded peribronchiolar lymphoid tissues with histiocytes and occasional multinucleate giant cells combined with broncho-interstitial pneumonia, PCV2 virus was demonstrated in only 64% of lungs with lesions of PMWS. These findings suggest that histopathology alone is non-specific for PMWS in lungs i.e. lesions of mycoplasmosis may resemble those of PCV2 infection and lead to false positives for PCV2 based only on histopathology. Alternatively, PCV2 infection may have caused the lesions, but the lesions remain longer than did detectable PCV2. There were similar findings for both livers and kidneys. Granulomatous inflammation typical of PMWS was identified in 88% of livers and 69% of kidneys, yet PCV2 was identified in only 15% of livers and 35% of kidneys. Again this suggests that histopathology alone lacks specificity for diagnosis of PMWS in liver and kidney or that lesions caused by PCV2 remain longer than does virus. It has been recommended that a diagnosis of PMWS requires wasting disease, typical gross lesions and typical microscopic lesions in which PCV2 is demonstrated.[23] The intracytoplasmic inclusion bodies observed in macrophages are unique and provide strong evidence for PCV2 infection, but in this study were observed in only 21% of PCV2-postive pigs. This suggests that methods such as in-situ hybridization or immunohistochemistry are needed to confirm PCV-2 in lesions. Lesions typical of PMWS were most commonly demonstrated concurrently with PCV2 in enlarged lymph nodes (82% of pigs) and ileum (82% of pigs), suggesting that these tissues would be the most useful for diagnostic testing. Although all of these pigs fulfill the aforementioned criteria for a positive diagnosis of PMWS, this study suggests that the financially significant wasting disease reported on these 17 farms cannot be attributed solely to PMWS. Twenty-six of the 34 pigs had concurrent diseases known to reduce growth rate; 22 had enzootic pneumonia, 2 had enzootic pneumonia and PRRS and the remaining 2 had septicemic salmonellosis. It is unclear what proportion of the wasting disease in each pig was caused by PMWS.

Twelve pigs originated from farms with a primary complaint of diarrheal disease and wasting. We demonstrated granulomatous inflammatory lesions with lymphoid depletion in the ileums of all pigs as well as lesions in other portions of the gastrointestinal tract in many pigs. However, the lesions were not obviously more severe in the pigs with diarrhea than in those without diarrhea. We found no other infectious causes of diarrhea. It is unclear whether PCV2 causes diarrhea.

Recent studies have suggested that PCV2 alone will not cause wasting disease, but concurrent infections of PCV2 and PPV or PRRSV will result in PMWS.[1, 15, 24] In this study, PAV was the most common co-infecting virus with PCV. Although PAV could be an inconsequential endemic viral infection, it is possible that PAV could serve as a co-factor enhancing replication of PCV2 in a fashion similar to PPV or PRRSV.

Objective 4: Comparison of the genomic sequence of contemporary PMWS and CT isolates and a historic CT isolate of PCV.

The sequences of the entire genomes of 4 PCV2 isolates from 4 pigs with PMWS originating from different farms in this study (PMWS-PCV-P1, -P2, -P3 and –P4), 2 recent PCV2 isolates associated with CT (CT-PCV-P5 and -P6),[25] and 1 PCV isolate of unknown type associated with CT in the late 1960s (CT-PCV-P7)[13] were compared with one another, with the first sequenced PCV2 isolate[10] and with the PK-15 PCV1 isolate.[17] Genomes of PMWS-PCV-P1, -P2, and -P4 were 1768 nt long, whereas PMWS-PCV-P3 was 6 nt shorter than the rest of PMWS-PCV isolates due to a 6-nt deletion between 820 to 825 nt. All the PMWS origin PCV2 isolates had an overall 99% nt sequence identity with each other. The coding strand, number of amino acids, and the location of each ORF in the genomes of all PCVs were determined. The amino acid sequence of ORF1 was highly homologous (approximately 99% homology at amino acid level) among all PMWS-PCV isolates. The ORF2 had more amino acid changes than ORF1 among PMWS-PCVs, but still had an approximately 97% homology. Open reading frames 3, 4, 7, and 8 were identical among PMWS-PCV isolates and there were only few changes in the rest of ORFs. The ORF5 of PMWS-PCV-P4 and ORF10 of PMWS-PCV-P3 were 54 and 26 amino acids longer than their counterpart in the rest of our PMWS-PCV isolates.

The recent CT-PCV isolates (CT-PCV-P5 and -P6) were both 1768 nt long and had approximately 99% nt sequence identity. Interestingly, these CT-PCV isolates also demonstrated ≥ 99% nt sequence identity with the PMWS-PCV isolates. The genomes of PMWS-PCV-P1 and CT-PCV-P5 were identical even though the farms-of-origin were located several hundred miles apart and did not share a common origin of stock. Both PMWS-PCV and new CT-PCV genomes encoded 11 potential ORFs.

The genome of the old CT-PCV (CT-PCV-P7) was 1759 nucleotides long and shared only approximately 72% nt sequence identity with PMWS-PCVs and both new CT-PCVs, but shared 98% nt sequence identity with PK-15-PCV. Thus, the old CT-PCV (CT-PCV-P7) is a PCV1. The genome of CT-PCV-P7 encoded 11 potential ORFs and the amino acid sequences of all ORFs were highly homologous to those of PK-15-PCV.

These findings indicate that all isolates of PCV2 from pigs with PMWS are nearly identical, suggesting that immunization with a vaccine derived from a single strain of PCV2 could potentially confer broadly cross-protective immunity. More work is needed to develop and evaluate the efficacy of vaccines. We also discovered that both PCV1 and PCV2 are associated with CT. More work is needed to determine whether PCV1 and/or PCV2 causes CT. Comparison of the genomic sequences between PCV2 isolates from PMWS and CT demonstrated no consistent differences. In fact, isolates from 1 pig with PMWS and 1 pig with CT were identical. This suggests that there are no

subtle differences in PCV2 isolates that might be responsible for differing tissue tropism or other virulence factor that might explain the differing disease manifestations between PMWS and CT. It also suggests that a monovalent PCV2 vaccine might protect against PMWS and PCV2-associated CT.

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