RESEARCH REPORT
SWINE HEALTH

Title: Assessment of viral load in clinical and subclinical pigs naturally infected with the novel PCV2b: implications for the control & prevention of PMWS/PCVD – **NPB #06-077**

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II. Industry Summary

After its discovery in the mid-1990's postweaning multisystemic wasting syndrome (PMWS) was noted only sporadically in North America for about a decade. However, since late 2004, the porcine circoviral diseases (PCVD) including PMWS have resulted in severe epidemics in various regions throughout North America, and continue to threaten the competitiveness of the North American swine industry. The rise in PCVD in N.A. since 2004 coincides with the isolation of a novel PCV2 strain referred to as PCV2-321 or PCV2b. Based on the near simultaneous emergence of this novel PCV2b and epidemics causing severe morality, some speculate that PCV2b is of enhanced virulence. The objectives of this study were to compare the amount of PCV2 in the tissues and sera of WASTING and HEALTHY pigs from 2 farms infected with PCV2b, and compare to UNAFFECTED pigs originating from a farm with no prior history of PMWS/PCVD. Secondly, PCV2 load in tissues, measured by quantitative PCR, was correlated with the severity of microscopic lesions and PCV2 staining intensity. Ten WASTING and 10 age-matched HEALTHY cohorts from each of two farms, and 10 UNAFFECTED pigs from a PCV2 infected farm with no prior history or diagnosis of PCVD were used in this experiment. From each pig, gross lesions were assessed; sera and multiple tissues were collected. Microscopic lesions suggestive of PCVD, and PCV2 staining intensity were scored (0-3) in all tissues by independent board certified pathologists. Levels of PCV2 DNA (viral load) were measured by quantitative PCV2 PCR (qPCR) in all tissues and sera. The highest viral load was found in WASTING pigs, and across all tissues. By contrast, the lowest PCV2 load was found in UNAFFECTED pigs from the barn with no prior history of PCVD/PMWS. PCV2 load in UNAFFECTED pigs was significantly lower than in HEALTHY pigs from farms suffering PCVD. Thus, in farms affected with PCVD/PMWS "WASTING" and visually "HEALTHY" pigs may be appropriately termed "clinical", "pre-clinical", whereas healthy pigs in UNAFFECTED farms may be appropriately termed "sub-clinical". Viral load, as measured by qPCR, was strongly correlated with PCV2 staining intensity and microscopic lesions characteristic of PCVD. Although the diagnosis of PCVD in individual animals requires microscopic examination and PCV2-specific staining of multiple tissues, qPCR is suited for population based monitoring of live animals, for example, the monitoring of control or vaccination programs. Sera, gluteal (ham) muscle, or inguinal lymph node are all appropriate

diagnostic samples to submit for the monitoring of PMWS/PCVD in commercial nursery and finisher pigs. Finally, the results of this project indicate that the biological relevance of PCV2 genotypes (2a, 2b) needs to be further clarified. The simultaneous presence of both PCV2a and 2b in UNAFFECTED pigs from a farm with no history of PMWS/PCVD implies that PCV2b is of no greater virulence than PCV2a. Although PCV2 DNA sequencing is of great academic interest, its value for commercial farms is less obvious.

III. Abstract

The PCVD/PMWS epidemics experienced in various regions throughout North America since late 2004 pose a significant threat to the entire North America swine industry, and coincide with the isolation of a novel PCV2 strain referred to as PCV2-321 or PCV2b. Regardless of the PCV2 strain and co-factor(s) involved, we proposed that PMWS/PCVD control is dependent on reducing and maintaining low PCV2 viral load. The objectives of this study were to compare the amount of PCV2 in the tissues and sera of WASTING and age-matched HEALTHY pigs from 2 farms infected with PCV2b, and compare to age-matched UNAFFECTED pigs originating from a farm with no prior history of PMWS/PCVD. Microscopic lesions suggestive of PCVD, and PCV2 staining intensity were scored in all tissues, and the levels of PCV2 DNA were assessed by quantitative PCV2 PCR (qPCR) in all tissues and sera. The highest viral load was found in WASTING animals. By contrast, the lowest viral load was found in UNAFFECTED pigs from a barn with no prior history of PCVD/PMWS. Viral load, as measured by qPCR, was strongly correlated with PCV2 staining intensity, and microscopic lesions associated with PCVD. The simultaneous presence of both PCV2a and 2b in UNAFFECTED pigs from a farm with no history of PMWS/PCVD implies that PCV2b is of no greater virulence than PCV2a. Thus, the biological relevance of PCV2 genotypes (2a, 2b) needs to be further clarified.

The results of this study will enhance the diagnostic capability of North America, specifically the interpretation of quantitative PCR. Moreover, the results will enhance the on-farm testing protocols used to assess control and prevention programs and PMWS/PCVD risk. By objectively evaluating the level of PCV2 load in tissues and sera by qPCR, diagnosticians will more accurately assess the biological significance of PCV2 infection in subclinically and pre-clinically affected live pigs and tissues. Moreover, this research has identified the tissues most suited for population based studies on live pigs, for example, monitoring control or vaccination programs.

IV. Introduction

After its discovery in Canada in the mid-1990's postweaning multisystemic wasting syndrome (PMWS) was noted only sporadically in North America for about a decade. However, since late 2004, the porcine circovirus diseases (PCVD) including PMWS have resulted in severe epidemics in various regions throughout North America, and continue to threaten the competitiveness of the North American swine industry. Many recent herd outbreaks in North America coincide with the isolation of a novel PCV2 strain, first identified by restriction fragment length polymorphism (RFLP) analysis as PCV2-321,[1] and more recently termed PCV2b.[2] Based on the near simultaneous emergence of this novel PCV2b and epidemics causing severe morality in affected North American herds, some speculate that PCV2b is of enhanced virulence.

The cause and epidemiology of the PCVD have been extensively researched and debated. While PCV2 infection is clearly a necessity and is the only virus consistently recovered from PWMS cases,[3-5] other co-factors are necessary to trigger or induce disease. These co-factors may include concurrent infections with other pathogens such as PRRS, mycoplasma, swine influenza, and parvovirus; immune stimulation or vaccination, and the absence of good production practices. However, virtually all commercially raised pigs are subclinically infected with low levels of PCV2,[6, 7] yet remain healthy and do not develop disease. By contrast, very high levels of PCV2 can be measured in the tissues of affected animals and are correlated with the severity of clinical signs and

histological lesions.[8] Thus, increasing viral load appears to be a critical step in the development of severe clinical disease.

V. Objectives:

The objectives of this study were to: 1) compare the amount of PCV2 in the tissues and sera of WASTING and HEALTHY pigs of 2 farms infected with PCV2b, and compare to UNAFFECTED pigs originating from an unaffected farm; 2) correlate the amount of PCV2 virus in tissues with the severity of microscopic lesions and PCV2 staining intensity; 3) determine the most appropriate diagnostic samples to submit from live pigs for the assessment of PCVD/PMWS in populations. An additional objective, to compare the viral load in pigs infected with PCV2a versus PCV2b was planned, based on our initial understanding of the PCV2 genotypes in each farm obtained through pre-existing diagnostic RFLP. However, fulfilling this objective was not possible, due to the unexpected PCV genotype results obtained from each farm during the experiment.

VI. Materials and Methods:

Pigs and farms: This was a descriptive study that compared severely affected (WASTING) pigs to age-matched healthy (HEALTHY) cohorts originating from two farms (A & B), and to unaffected (UNAFFECTED) pigs from a PCV2 infected farm with no prior history or diagnosis of PCVD (Farm C). Farms A & B were both 1200 sow farrow to finisher farms with excellent health status. Both farms were owned by the same company, and had similar management. Farm C was unrelated to A & B, but used similar PIC genetics and was of similar high health status. PCV2 had never been diagnosed in the farm, but the virus was assumed to be actively circulating in nursery and/or grower pigs. *PCV2 genotyping:* Early in the experiment, ORF 2 sequencing (Prairie Diagnostic Services [PDS], Saskatoon) was undertaken on the lymph nodes of 2 WASTING pigs from each of Farms A & B to confirm the PCV2 genotypes were consistent with previous RFLP diagnostics. During the experiment, the lymph nodes from two healthy Farm C pigs were also sequenced using identical methodology. The raw sequence data was compared among isolate, with emphasis on the sma*I* restriction site, which can be used to clearly differentiate PCV2a and 2b. *Sample collection:* From each of Farms A and B, 10 WASTING pigs typical of PCVD were identified in the nursery and grower barns (50:50), along with an equal number of age matched HEALTHY cohorts. From Farm C, 10 aged-matched healthy pigs of similar genetics and in good body condition were also selected (UNAFFECTED). Unlike Farms A and B, some of the selected pigs from Farm C had small incidental umbilical hernias or were ridglings. These pigs were preferentially selected based on their lower economic value, with the assumption that PCV2 levels would not be impacted by the presence of a hernia or retained testicle. All pigs were uniquely ear tagged at selection, euthanized by captive bolt, and post-mortemed. About 10 mL blood was collected from each prior to euthanasia, and the serum was separated and frozen. During necropsy, all abnormalities were recorded and multiple tissues were collected and preserved appropriately. *Assessments:* The severity of microscopic lesions and intensity of PCV2 specific staining was assessed independently by two Board Certified pathologists who did not have any knowledge of the experimental design or pig group allocations. In all tissues and sera, the amount of PCV2 DNA (viral load) was assessed using an in-house PCV2 quantitative PCR assay (qPCR) by the Prairie Diagnostic Services (PDS), Saskatoon, Saskatchewan. *Statistical analysis:* The frequency of gross carcass abnormalities was compared between the health status groups using the Fisher's exact test. The viral load, severity of microscopic lesions and intensity of PCV2 staining in tissues were compared among barn and health status group using the Kruskal-Wallis analysis of variance. The correlations between viral load and microscopic lesion severity, and viral load and PCV2 staining intensity were computed using the Spearman rank correlation coefficient. The sensitivity and specificity of qPCR in serum, pooled lymph nodes (bronchial, superficial inguinal, mesenteric), and gluteal (ham) muscle were determined using Receiver Operating Characteristic (ROC) curves using WASTING as a classification variable.

VII. Results:

Gross lesions: The gross lesions by farm and health status are presented in Tables 1-3. Compared to HEALTHY pigs, WASTING pigs on post-mortem examination demonstrated significantly more frequent ($P < 0.05$ for all):

- depletion of the thymus gland,
- anteroventral lung consolidation (indicative of bronchopneumonia),
- hyper-inflated lungs (indicative of interstitial pneumonia),
- fluid-filled abdominal cavities,
- dilated fluid-filled large intestines (indicative of a severe diarrhea).

Compared to UNAFFECTED pigs, WASTING pigs demonstrated significantly more frequent lymph node enlargement ($P = 0.001$), and numerically more frequent hyper-inflated lungs ($P = 0.06$). Compared to UNAFFECTED pigs, HEALTHY pigs demonstrated numerically more frequent lymph node enlargement ($P = 0.06$).

Microscopic lesions: Microscopic lesions in all WASTING and some HEALTHY pigs were characteristic of PCVD/PMWS, and a detailed diagnostic examination of tissues did not indicate the involvement of other swine diseases such as mycoplasma pneumonia, PRRS (all farms were PRRS free), pleuropneumonia, or swine influenza (data not shown). WASTING pigs had significantly less pronounced lymph node germinal centers, and more severe lymphocyte depletion than HEALTHY and UNAFFECTED pigs ($P < 0.05$ for both), suggesting the presence of immune suppression in WASTING pigs. Interstitial pneumonia was noted in all groups, but was significantly severe in WASTING compared to UNAFFECTED pigs ($P < 0.05$).

Viral load: Across all tissues and sera, the viral load (DNA copies) and PCV2 staining intensity were significantly higher in WASTING compared to HEALTHY and UNAFFECTED pigs ($P < 0.05$ for all). There were no statistical differences in viral load and PCV2 staining intensity between Farm A and B. Viral load in most lymph nodes, as well as kidney, liver, lung and heart, but not sera, was significantly higher in HEALTHY compared to UNAFFECTED pigs ($P < 0.05$ for all). The severity of lymphoid depletion in lymph nodes was positively correlated with viral load ($r = 0.68$; $P < 0.05$). Moreover, viral load was strongly and positively correlated with immunoperoxidase staining intensity in lymph node, spleen, Peyer's Patch and lung ($0.75 < r < 0.84$; $P = 0.0$ for all). In most tissues examined, generally low levels of PCV2 DNA were detected in tissues in which there was no immunoperoxidase staining. For sera, pooled lymph nodes and gluteal muscle, the area under the curve (AUC) generated using ROC curve analysis were 0.959, 0.938, 1.00 respectively, indicative an very high sensitivity and specificity for each sample over a wide range of qPCR cutpoints.

Assessment of PCV2 genotypes: Genotype PCV2b was identified in the lymph nodes of two WASTING pigs from Farm A, consistent with pre-existing laboratory data. PCV2b was also identified in the lymph nodes of two WASTING Farm B pigs, contrary to the pre-existing laboratory results that had only indicated the presence of PCV2a on the farm. Additional Farm B samples were re-submitted with consistent results. By contrast, both PCV2a and 2b were identified in the lymph nodes of the UNAFFECTED pigs originating from Farm C.

VIII. Discussion:

In keeping with the objectives of this experiment, we compared the viral load in the tissues and serum of WASTING and HEALTHY pigs from affected farms, to UNAFFECTED pigs from an unaffected farm, and found the highest viral load in WASTING animals. This finding agrees with previous research[8-11] which has demonstrated high viral load in pigs with PCVD/PMWS. However, this project is novel in that we measured viral load in farms infected with the novel Canadian PCV2b. Moreover,

the lowest viral load was found in UNAFFECTED pigs from a barn with no prior history of PCVD/PMWS. More specifically, viral load in UNAFFECTED pigs was significantly lower than in HEALTHY pigs from affected farms. Under the conditions of this experiment, WASTING, HEALTHY, and UNAFFECTED pigs are appropriately described as “clinical”, “pre-clinical” and “sub-clinical” respectively.

Viral load, as measured by qPCR, was strongly correlated with PCV2 staining intensity, and microscopic lesions associated with PCVD. Although the diagnosis of PCVD in individual animals is reliant on microscopic examination and immunohistochemistry (IHC) of multiple tissues, quantitative PCR is more suited than IHC for population based monitoring of live animals, for example, evaluating the effectiveness of control or vaccination programs. Sera, gluteal (ham) muscle, or inguinal lymph node are all appropriate diagnostic samples to submit for the ante-mortem diagnosis of PMWS/PCVD in commercial nursery and finisher pigs.

Unfortunately, because PCV2b was identified in both Farm A and B, we were unable to compare the relative viral load and virulence of the PCV2a and 2b genotypes. Moreover, DNA sequencing of the PCV2 isolates obtained from the UNAFFECTED Farms C pigs yielded surprising results. More specifically, the simultaneous presence of both PCV2a and 2b in the same tissue of UNAFFECTED animals from an unaffected farm was unexpected, and implies that PCV2b is of no greater virulence than PCV2a, and that PMWS/PCVD is not made more severe by dual PCV2a/2b infection. Thus, while PCV2 DNA sequencing is of great academic interest, our results suggest its value for commercial diagnostic applications is less obvious. Although virulence differences have been previously demonstrated between PCV2 strains,[12] there is still insufficient scientific evidence to conclude that the novel 2b strain is causally associated with the present PCVD epidemic in North American.

IX. References

1. Carman S, McEwen B, DeLay J, T. vD, Lusis P, Cai H, Fairles J. Porcine circovirus-2 associated disease in swine in Ontario (2004-2005). *Canadian Veterinary Journal.* 2006;47:761-762.
2. Gagnon CA, Tremblay D, Tijssen P, Venne M, Houde A, Elahi SM. The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada. *Canadian Veterinary Journal.* in press.
3. Krakowka S, Ellis JA, McNeilly F, Ringler S, Rings DM, Allan G. Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). *Veterinary Pathology.* 2001;38:31-42.
4. Ellis JA, Bratanich A, Clark EG, Allan G, Meehan B, Haines DM, Harding J, West KH, Krakowka S, Konoby C, Hassard L, Martin K, McNeilly F. Coinfection by porcine circoviruses and porcine parvovirus in pigs with naturally acquired postweaning multisystemic wasting syndrome. *Journal of Veterinary Diagnostic Investigation.* 2000;12:21-27.
5. Allan GM, McNeilly F, Krakowka S, Ellis J, Charreyre C, Botner A, Nauwynk H, McCullough K, Wallgren P, Caprioli A. Porcine circovirus diseases: 1996-2004 and beyond! *Pig Journal.* 2004;54:139-145.
6. Larochelle R, Magar R, D'Allaire S. Comparative serologic and virologic study of commercial swine herds with and without postweaning multisystemic wasting syndrome. *Canadian Journal of Veterinary Research.* 2003;67:114-120.
7. Allan GM and Ellis JA. Porcine circoviruses: a review. *Journal of Veterinary Diagnostic Investigation.* 2000;12:3-14.
8. Krakowka S, Ellis J, McNeilly F, Waldner C, Allan G. Features of porcine circovirus-2 disease: correlations between lesions, amount and distribution of virus, and clinical outcome. *Journal of Veterinary Diagnostic Investigation.* 2005;17:213-222.

9. Brunborg IM, Moldal T, Jonassen CM. Quantitation of porcine circovirus type 2 isolated from serum/plasma and tissue samples of healthy pigs and pigs with postweaning multisystemic wasting syndrome using a TaqMan-based real-time PCR. *Journal of Virological Methods.* 2004;122:171-178.
10. Olvera A, Sibila M, Calsamiglia M, Segales J, Domingo M. Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs. *Journal of Virological Methods.* 2004;117:75-80.
11. Ladekjaer-Mikkelsen AS, Nielsen J, Stadejek T, Storgaard T, Krakowka S, Ellis J, McNeilly F, Allan G, Botner A. Reproduction of postweaning multisystemic wasting syndrome (PMWS) in immunostimulated and non-immunostimulated 3-week-old piglets experimentally infected with porcine circovirus type 2 (PCV2). *Veterinary Microbiology.* 2002;89:97-114.
12. Opriessnig T, McKeown NE, Zhou EM, Meng XJ, Halbur PG. Genetic and experimental comparison of porcine circovirus type 2 (PCV2) isolates from cases with and without PCV2-associated lesions provides evidence for differences in virulence. *Journal of General Virology.* 2006;87:2923-2932.

Table 1. Gross lesions in 20 age matched pigs originating from Farm A

Age matched pair	HEALTHY PIGS - NURSERY		WASTING PIGS - NURSERY	
	ID	**Gross lesions**	**ID**	**Gross lesions**
1	43	Scruffy, pericardial adhesions to thoracic wall; fluid in pericardial sac	47	Mesocolonic edema; mild splenic marginal congestion; mild interstitial nephritis, severe pulmonary edema with intermittent consolidation of cranial lobes; systemic compartmental edema (thorax, abdomen etc)
2	16	NAF	1	Ulcerative skin lesions face & ears; moderately enlarged inguinal lymph nodes; few small infarcts on left kidney; caudodorsal interstitial pneumonia; cranioventral bronchopneumonia (50%); enlarged bronchial LN; mild ascites
3	5	10-15 ml fluid in pericardial sac	29	Enlarged inguinal LN; hydrothorax; pneumonia; bronchopneumonia of anterior and middle lobes
4	14	Infarcts on margins of spleen	50	Atrophy of liver margins; markedly small thymus; small kidneys; bronchopneumonia of middle lung lobes (8%); enlarged bronchial LN; mild fibrin deposition in abdomen; necrotizing colitis
5	15	Enlarged systemic LN and thymus	45	Mild atrophy of liver margins; depleted Peyer's Patch; bronchopneumonia (20%) of right middle lobe; copious yellow tinted fluid in abdomen (ascites)
Pair	**HEALTHY PIGS - GROWER**		**WASTING PIGS - GROWER**	
6	49	Hemorrhage under capsule of bronchial LN; 3 mL fluid in pericardial sac	35	Emaciated; consolidated middle lung lobes; small amounts fibrin in abdomen; small thymus
7	22	Bronchopneumonia (10%) of right middle lung lobe; hemorrhagic bronchial LN	19	Pale, enlarged inguinal and bronchial LN; diffusely scatter grey foci on kidneys, dilated colon with prominent lymphoid follicle hyperplasia
8	46	NAF	37	Enlarged inguinal LN; mild splenic marginal congestion; consolidation of middle and margin of caudal lobe; dilated and fluid filled intestines
9	12	Congestion of margins of spleen; 1% lung lesion of middle lung lobe; hemorrhagic bronchial LN	10	Mild atrophy of liver margins; enlarged, pale kidneys; small thymus; interstitial pneumonia; mesocolonic edema; ascites; necrotizing colitis
10	7	NAF	40	Small infarcts margins of spleen; slightly hyper-inflated lung; dilated fluid filled colon; colonic lymphoglandular hyperplasia

Legend: NAF = no abnormal findings; LN = lymph nodes

Table 2. Gross lesions in 20 age matched pigs originating from Farm B.

Age match pair	HEALTHY PIGS - NURSERY		WASTING PIGS - NURSERY	
	ID	**Gross lesions**	**ID**	**Gross lesions**
1	36	Slightly enlarged systemic LN	21	Mild marginal congestion of spleen; pale kidneys; depleted thymus; hyper-inflated lung with prominent lobular pattern; anteroventral consolidation (20%); dilated, fluid filled spiral colon
2	113	Slightly enlarged systemic LN; slight depression of tip of middle lung lobe	90	3-5 mm brown/red foci on tip and margin of ear, and flank; marginal congestion of spleen (50% affected); liver mottled dark red, depressed; small kidneys; depleted Peyer's Patch and thymus; bronchopneumonia with pleural edema; enlarged bronchial LN; ascites with flecks of fibrin.
3	30	NAF	26	Very enlarged systemic LN; hyper-inflated caudal lung lobes; cranioventral bronchopneumonia
4	31	Slightly enlarged and edematous systemic LN	4	Small 0.5 cm hemorrhage on pole of inguinal LN; enlarged, edematous mesenteric LN; depleted thymus; hyper-inflated lung with prominent lobular pattern; few fibrin tags in abdomen; mild mesenteric edema
5	120	Enlarged right inguinal LN; large thymus; inflammation on serosal surface of colon, slight excess pericardial fluid	86	Severe depletion of Peyer's Patch; depleted thymus; dilated fluid filled colon; consolidation (5%) middle lung lobe with exudates on cut surface
Pair	**HEALTHY PIGS - GROWER**		**WASTING PIGS - GROWER**	
6	6	Some pallor of liver margins; mottled tan/red kidneys with few pinpoint white foci; clearly visible Peyer's Patch, large thymus	24	Poorly visible inguinal LN, enlarged systemic LN; multifocal random interstitial infiltrate in both kidneys; depleted thymus
7	33	Moderate marginal congestion of spleen; enlarged globus heart, dilated right ventricle (valves normal); large thymus	17	Unthrifty; enlarged LN throughout carcass; mild decrease in liver mass; multifocal random, interstitial infiltrate in both kidneys; generalized petechiation on lungs
8	44	Enlarged LN throughout; few small foci bilaterally	3	Enlarged inguinal LN; multiple diffuse white pinpoint foci on kidneys; depleted thymus & Peyer's Patch; hyper-inflated lung; ascites; full stomach
9	102	Few scattered white foci on kidneys; clear fluid in pericardial sac	25	Enlarged systemic LN; mild bilateral kidney enlargement with diffuse interstitial infiltration; mild peritoneal, pleural, pericardial effusions with a few proteinatious flakes; multifocal 2 mm infiltrates in renal cortex
10	89	Multiple enlarged LN; possible tonsillitis (edema with subtle white foci on ventral surface); few white spots on kidney	108	Enlarged systemic LN; congestion of splenic margins, thinning of liver margins; subtle scattered white foci on kidneys; grey mottling throughout lung; prominent colonic lymphoglandular follicles

Table 3. Gross lesions in 10 healthy pigs originating from Farm C (unaffected).

Age	HEALTHY PIGS - NURSERY	
	ID	**Gross lesions**
6.5 wks	155-10 (9)	Rupture (golf ball); multifocal 0.5-1.0 cm haemorrhagic lesions in left middle lung lobe in lobular pattern; possible thickened ileum
6.5 wks	155-15 (48)	Rupture (golf ball); haemorrhagic sternal LN; multiple dark red foci (1cm sq) on ventral portion of cranial lung lobe and dorsal aspect of left lung; red multifocal coalescing areas throughout serosal surface of kidney; colonic lymphoglandular hyperplasia; dark feces possible melena.
7.5 wks	154-14 (18)	Rupture (baseball); numerous lacerations/excoriations on trunk, flank and abdomen; 3 mm areas of congestion on splenic margin; colonic lymphoglandular hyperplasia
7.5 wks	154-3 (28)	Small 1 cm patches of dark red on caudoventral lung
Age	**HEALTHY PIGS - GROWER**	
8.5 wks	140-12 (39)	Rupture (small)
9.5 wks	139-14 (42)	Ridgling; colonic lymphoglandular hyperplasia
10.5 wks	147-14 (34)	Mild marginal splenic congestion; liver pale in colour; small thymus; possibly bronchopneumonia
11.5 wks	148-16 (41)	Rupture (softball); cyst in right kidney; focal white spot on kidney; reddish discolouration on inside of thymus.
13.5 wks	142-13 (13)	Ridgling; multifocal 5mm spots throughout liver
14.5 wks	145-10 (11)	Rupture (baseball); multifocal red spot(s) on liver