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Assessment of viral load in clinical and subclinical pigs naturally infected with the novel PCV2b: Implications for the control and prevention of PMWS/PCVD

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Introduction

After its discovery in Canada in the mid-1990's post-weaning 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 epidemics causing severe morality, and this novel PCV2b 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.

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.

Materials and methods

Pigs and farms

This was a descriptive study that compared severely affected (WASTING) pigs to age-matched healthy (HEALTHY) penmates 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 and 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.

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 penmates. 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 animals 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 prior to euthanasia, and the sera were 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 in a blinded manner by pathologist. 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. The PCV2 isolated from 2 pigs from each farm were DNA sequenced by PDS, and subsequently identified as PCV2a or P2b.

Statistical analysis

The frequency of gross carcass abnormalities was compared between health status 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 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.

Results

Gross lesions

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 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 ($P < .05$ 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 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.

PCV2 genotyping

The PCV2 isolates from WASTING pigs from Farms A & B were PCV2b, whereas both PCV2a and 2b were isolated from UNAFFECTED pigs from Farm C.

Discussion

In keeping with the objectives of this experiment, we have compared the viral load in the tissues of WASTING and HEALTHY pigs from affected and non-affected farms infected with a novel PCV2b isolate, and found the highest viral load in WASTING animals. This finding agrees with previous researchers[8-11] who have demonstrated high viral load in pigs with PCVD/PMWS. However, this project is novel in that we measured viral load in farms infected with 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, monitoring 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.

DNA sequencing of the PCV2 isolates obtained from Farms C yielded surprising results. More specifically, the simultaneous presence of both PCV2a and 2b in animals from an unaffected farm was unexpected, and implies that PCV2b is of no greater virulence than PCV2a, nor is PMWS/PCVD made more severe by dual PCV2a/2b infection. 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 North American epidemic.

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