

The effect of a commercial vaccine and adjuvants on the prevalence of post-weaning multisystemic wasting syndrome and related circovirus lesions in a finishing barn

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Introduction

Post-weaning multisystemic wasting syndrome (PMWS) is an important emerging viral disease affecting nursery and grower pigs, which has recently been associated with porcine circovirus type 2 (PCV2) infections. PMWS has been reported in Europe, South East Asia, and the Americas^{1,2,3}. Although PCV2 has been present in pig populations since at least 1973, only recently have clinical signs been associated with its presence^{4,5}.

Clinical manifestations of PMWS include poor body condition with varying degrees of muscle wasting, dyspnea and enlarged lymph nodes; pallor, diarrhea, and jaundice are less frequent. Grossly, lesions in the lungs vary from failure to collapse and increased firmness to diffuse mottling, with areas of consolidation in the cranioventral lobes. Often, the affected pigs will have markedly enlarged lymph nodes with a white and homogenous cut surface^{5,1}. Histologically, PMWS can produce intracytoplasmic inclusion bodies in vacuolated mononuclear cells, lymphoid depletion/lymphocytolysis of all lymphoid organs and tissues, and patchy to diffuse interstitial pneumonia with lymphocytic-histiocytic infiltrates with variable numbers of multinucleated syncytial cells⁵. The organ system affected and the morbidity/mortality rates can vary among infected animals and herds⁶.

Although PCV2 can act as a primary disease agent⁷ pigs infected solely with PCV2 often only develop mild or subclinical illness. Concurrent infections with other pathogens such as Porcine Parvovirus (PPV) or Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) can predispose pigs to a more severe PCV2-related wasting syndrome¹¹. In field conditions, tracing the natural history of PMWS is difficult because of inconsistencies in clinical signs, pathologic lesions⁶ and the varied nature of concurrent infections.

Recently, Krakowka et al. (2001) were able to demonstrate that adjuvant-draining lymph nodes from a PCV2-infected pig were immunohistochemically more strongly positive for PCV2 antigen than nodes located farther from the injection site.

The authors speculated that an introduced immunostimulus combined with a pre-existing infection with PCV2 may result in fulminant PMWS.

The use of commercial vaccines with adjuvants is common practice in swine production. It was the intent of this study to examine the effects of immunostimulation combined with a pre-existing infection with PCV2 under field conditions. The mortality rate in a finishing barn in Prince Edward Island, Canada was recorded and analyzed. The barn had recently suffered from an higher prevalence of PMWS following implementation of a vaccination program for *Mycoplasma hyopneumoniae* using RespiSure-ONE™, Pfizer Animal Health, NY, USA. To properly evaluate the possible impact of vaccination and non-specific immunostimulation on the development of clinical PMWS in field conditions, several immunostimulants were tested on the following fill.

Materials and methods

The study was initiated on April 8, 2002 and terminated June 7, 2002. A total of 725 56-day-old pigs were placed in an all-in/all-out feeder barn. The pigs originated from a single source high-health herd free of PRRSV, APP, and TGE/PRCV and were born within 10 days of each other. The pigs were weighed and divided into 5 treatment groups; males and females were equally allocated to each group, and pens were randomly allocated to each treatment as follows:

- Treatment 1 pigs received an IM injection of a one

- dose commercial vaccine using RespiSure-ONE™;
- Treatment 2 pigs received an IM injection of a commercially available oil adjuvant, Emulsigen® (MVP Laboratories, Ralston Neb. USA);
 - Treatment 3 pigs received an IM injection of a commercially available aluminum hydroxide adjuvant, Alhydrogel™ (HCI Biosector, Denmark);
 - Treatment 4 pigs received a placebo IM injection (physiological saline);
 - Treatment 5 pigs received no injections.

Additionally, equal numbers of pigs from each treatment group were placed in either pens with straw bedding or a slatted floor.

Post-mortem examinations

Necropsies were performed on all pigs that died during the experiment, i. e. after the treatments were administered and before slaughter. Body condition was recorded to evaluate wasting. In addition, a sample of lung and mesenteric lymph node were submitted for virus isolation. Tissue samples from the brain stem, heart, liver, mesenteric lymph node, spleen, lung and small intestine were fixed in 10% buffered formalin and routinely processed for histologic evaluation⁸.

A complete histopathology report was produced for every pig submitted for necropsy. Lesions were categorized by the presence of either 1) intracytoplasmic inclusions, 2) lymphoid depletion/lymphocytolysis of lymphoid tissues (mesenteric lymph node, spleen or Peyer's patch), or 3) interstitial pneumonia with syncytial cells.

Virology

Pieces of lung and mesenteric lymph node were collected, for fluorescent antibody test (PCV2 FAT) and Polymerase Chain Reaction (PCV2 PCR) for porcine circovirus type 2.

The PCV2 PCR was based on published and aligned sequences of porcine circovirus type 2⁹. The PCR utilizes the forward primer from PCV2 nucleic acid sequence 1459 to 1481 and the reverse primer from nucleic acid sequence 1565 to 1586 giving a PCR product of 127 base pairs. Porcine lymph node with the characteristic intracytoplasmic inclusions of PCV2 was used as positive control.

Diagnosis of post-weaning multisystemic wasting syndrome

The definitive diagnosis of the PCV2-related wasting

disease is based on the detection of viral antigen and/or nucleic acid, characteristic histo-pathological lesions, and compatible clinical signs^{1,10}. In the present study, a pig was determined to have PMWS when it fulfilled all three of the following parameters:

1. Positive identification of the virus by FAT and/or PCR;
2. Gross signs of wasting (at least moderate loss of muscle mass, body fat stores);
3. Histological lesions, either intracytoplasmic inclusion bodies or both lymphoid depletion/lymphocytolysis (mesenteric lymph node, and/or spleen, and/or Peyer's patch) and interstitial pneumonia with syncytial cells.

Statistics

A one-way ANOVA test was used to compare the number of mortalities due to PMWS in each group and the possible effect of the start-weight on the development of PMWS. A chi-square test evaluated the correlation between PMWS and gender and type of flooring (bedding versus slatted). Significance was determined at $P < 0.05$.

Results

A total of 30 pigs died during the experiment. Overall there was an average barn mortality rate of 3.0 %. Using the above definition, PMWS was determined to be the cause of death in 9 pigs, 3 pigs in treatment 1, 5 pigs in treatment 3 and 1 pig in treatment 5 (0.9% of the barn). Significant difference ($p = 0.035$) in the number of mortalities due to PMWS was found between treatment 3 (aluminum hydroxide) and treatment 2 (oil) and between treatment 3 and treatment 4 (saline). No significant difference ($p > 0.05$) was found between the remaining treatments. One pig in the control treatment developed severe PMWS.

Of the 9 PMWS positive dead pigs, 8 had been placed in pens with fully slatted floors, 1 in a pen with bedding ($p = 0.028$). Mean starting weights of PMWS and non PMWS pigs were significantly different ($p = 0.038$). Gender predisposition relative to diseased pigs produced a P -value of 0.101 on a chi-square test.

Discussion

A higher number of pigs treated with aluminum hydroxide developed PMWS than pigs treated with either saline or oil based adjuvant, suggesting that perhaps this immunostimulant may have facilitated the

development of clinical disease. However, when considering the fact that only 9 pigs developed PMWS, and they were distributed among 3 treatment groups, our findings should be confirmed in future studies. Higher numbers of deaths due to PMWS would have allowed us to more confidently interpret associations between groups. It is therefore more accurate to simply state that PMWS was present in pigs submitted to various treatments as well as in control pigs, and that there is probably a slightly higher risk of dying with PMWS associated with injecting aluminum hydroxide compared to saline or an oil adjuvant. More trials are needed to confirm this hypothesis.

In earlier studies^{7,11}, inoculation of PCV2 alone was reported to cause only mild disease while mortalities associated with PMWS were thought to require a concurrent viral infection¹¹. In this field study, pigs had a PCV2 infection and 9 out of 725 (1.24%) pigs developed severe lesions associated with PMWS. This finding is consistent with the more recent literature that acknowledges PCV2 as a disease agent capable of causing severe illness in field conditions¹². Interestingly, one of the pigs that developed the severe form of the disease was in the control treatment that was not iatrogenically immunostimulated.

Overall, our results are consistent with a recent Danish field study¹² in which both immunostimulated and non-immunostimulated PCV2-infected pigs developed clinical PMWS. Our study also appears to be consistent with previous suggestions that injecting immunostimulants could be responsible for the progression of a PCV2 infected pig to develop clinical PMWS⁶. There are probably other unknown factors involved in the development of PMWS in commercial operations, and clearly further studies are needed to identify possibly influential factors.

Fully slatted flooring (compared to deep bedding) and lower starting weights seemed to predispose pigs to PMWS in our study. Although PMWS was more frequently found in the fully slatted side, the affected pens were randomly located within that side in accordance to what has been reported in the literature⁴. Before concluding that floor type had an association with PMWS, it should be noted that pigs on the fully slatted floor had a smaller starting weight than those on the bedding. There may be confounding between floor type, starting weight and the risk of PMWS. In the previous fill there appeared to be no difference between PMWS risk in the two floor types. There was a trend

towards barrows having a higher risk of PMWS ($p=0.10$). To our knowledge, no other reports of the association between occurrence of PMWS and factors such as gender, floor type, and starting weights have been published.

The conditions in which the pigs were living are very similar to finishing barns throughout North America. It is possible that chemical immunostimulation does cause PMWS as Krakowka et al. (2001) speculated. However, it is difficult to extrapolate laboratory findings to a typical farm situation where pigs are naturally and chemically immunostimulated by environment and vaccination schedules. From the findings of this study, it appears that chemical immunostimulation, caused by vaccination and or adjuvants may have contributed to clinical PMWS in a situation where PCV2 was already associated with disease. More studies should be carried out to confirm this observation.

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Table 1: Allocation of treatments, prevalence rate, and number of pigs positive for PMWS.

Group	Treatment	PMWS cases	Total pigs in groups	Prevalence rate
1	Mycoplasma vaccine	3	172	1.7%
2	Oil adjuvant	0*	172	0.0%
3	Aluminum hydroxide adjuvant	5*	173	2.9%
4	Saline injection	0*	197	0.0%
5	Control	1	95	1.1%

* $P < 0.05$

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**Table 2:** The mean startweight of pigs with or without PMWS.

	PMWS	Non PMWS	P
Start weight	19.4 kg	22.3 kg	$P=0.040$

Table 3: Floor type and the prevalence of PMWS

	PMWS	Non PMWS	P
Bedded floor	1	425	
Slatted floor	8	382	$P=0.028$

Table 4: Gender and the prevalence of PMWS

	PMWS	Non PMWS	P
Gilts	3	480	
Barrows	6	316	$P=0.101$