Association of PCV2 antigen and antibodies with breed and post-mortem findings in PMWS suspicious pigs

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INTRODUCTION

Post-weaning multi-systemic wasting syndrome (PMWS) is a multi-factorial disease with porcine circovirus type 2 (PCV2) as necessary cause and mainly affects pigs aged 6-16weeks. The presence of other pathogens , environmental factors and/or genetic predisposition have been suggested to be involved in the onset of clinical signs. Affected pigs show wasting, paleness, hairiness, enlarged lymph nodes, scouring and often dyspnoea. PMWS in a herd is characterised by a significant and prolonged increase in mortality and morbidity. Over the last few years PMWS has transited from an epidemic to an endemic stage and the severity with which farms are affected varies considerably.

PCV2 has been associated with a number of pathological conditions of pigs, including porcine dermatitis and nephropathy syndrome, reproductive failure, porcine respiratory disease complex, proliferative and necrotising pneumonia and congenital tremor (Segales et al, 2004). The most common pathological findings include non-collapsed, tan-mottled lungs, pulmonary consolidation, enlargement of at least one lymph node , gastric ulceration of parts oesophagea, serositis, kidney with white foci and jaundice.

In 2008, the Royal Veterinary College (RVC) in collaboration with the British Pig Executive Ltd (BPEX) implemented a research project investigating risk factors affecting the severity of PMWS on affected farms. From April 2008 to March 2009, 145 farms in England have been recruited.

OBJECTIVES

The study presented here aimed at investigating the correlation of various post mortem findings with PCV2 virus titres and the presence of PCV2 antibodies.

The objectives were

- to describe and assess the frequency of common gross post-mortem findings in PMWS suspicious pigs in order to optimise future post-mortem protocols.
- to assess the correlation of post-mortem findings and PCV2 serum titres found in PMWS suspicious pigs.
- to assess the predisposition of different breeds for the development of severe clinical signs

MATERIAL & METHODS

Fifty-seven clinically PMWS suspicious pigs with low or moderate-severe wasting were necropsied and macroscopic post-mortem findings were recorded following a standard protocol that was developed based on findings of *Segales et al*, 2004 (table 1). The body condition scoring is illustrated in figure 1.

Samples of lymph nodes and tissues showing abnormality were collected and fixed in 10% buffered neutral formalin for future histo-pathological examination. Of all pigs serum samples were tested for the presence of antibodies to PCV2, *Mycoplasma Hyopneumoniae*, Actinobacillus Pleuro-Pneumoniae, Porcine Parvo Virus, and Porcine Respiratory and Reproductive Syndrome virus by ELISA and the presence of PCV2 antigen was determined by quantitative PCR.

The genetic background of pigs was assessed based on the breed of grand-parents and parents.

Table 1 - Protocol summary

Macroscopic findings	Categories
Body Condition	Normal/ Thin / Wasted (figure 1)
Paleness of the Skin	Yes / No
Skin more hairy	Yes / No
Non-collapsed, tan mottled lungs	Yes / No
Pulmonary consolidation	% of lungs
Pleurisy	Yes / No
Enlargement of Lymph Nodes	Traqueal / Mesenteric / Inguinal
Gastric ulceration parts	Normal / Moderate diffuse parakeratosis / Severe diffuse parakeratosis / Chronic diffuse ulceration
Serositis	In Joints / In thorax / In peritoneum
Kidney white foci	None / 1 kidney<10 focis/ 2 kidneys <10 focis / Both kidneys >20 focis
Kidney colour	Normal /Pale
Kidney size	Normal / Enlarged
Kidney capsule attached	Yes / No
Fat atrophy in heart	Yes / No
Jaundice	Yes / No
Liver fibrosis	Yes / No
Spleen enlarge	Yes / No
Large intestine comments	
Small intestines comments	

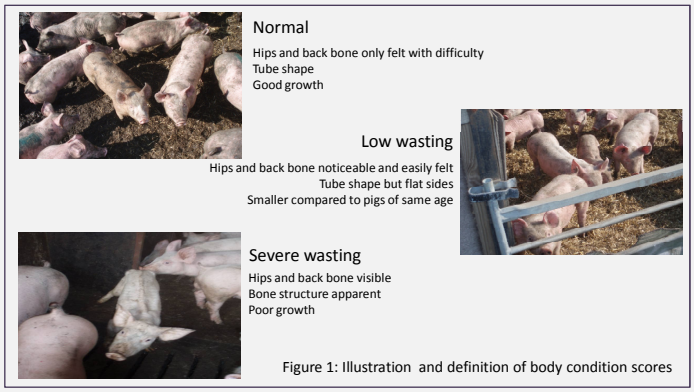


Figure 1: Illustration and definition of body condition scores

Data analysis

Statistical analysis to assess association between various variables and various outcomes was conducted using chi-square test, fisher's exact test, logistic regression and linear regression models with the software package Stata 9 (Statacorp, texas, USA).

RESULTS

Descriptive analysis PCV2 virus was detected by qPCR in 42 animals (71.7%, CI95%: 60.02 - 83.38), whereas the prevalence of PCV2 antibodies was 31.4% (CI95%: 19.35 - 43.45), laboratory results are shown in figure 2.

Severe wasting was observed in 39 (67%) of pigs. A summary of the post-mortem findings of all the animals, incl. stratified for PCV2 PCR positive and negative animals with their respective p-values is presented in the Figure 3. There was no significant association of any of the post-mortem finding and the virus load as determined by qPCR.

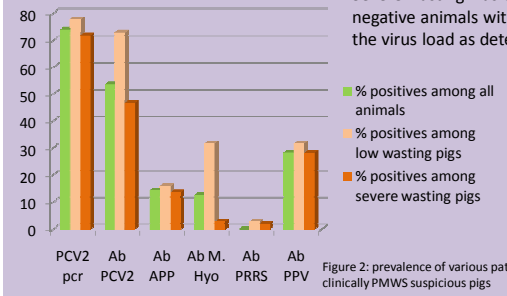
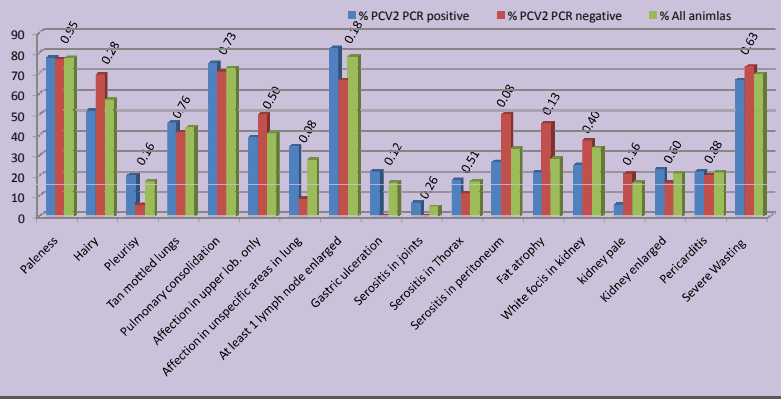


Figure 2: prevalence of various pathogens in clinically PMWS suspicious pigs

Figure 3 – Post-mortem findings incl. p-values



Correlation with outcome 'PCV2 PCR + and neg for PCV2 antibodies'

No significant association was found with any of the gross lesions examined. However the increase of 25% of Landrace in a pig resulted in significant lower odds to be PCR + and not having antibodies (OR 0.14, p<0.01). Higher odds were found for pig weighing less than 30 kg (OR 1.07, p=0.02).

Correlation with outcome 'severe wasting'

In univariate models the degree of wasting was strongly associated with the presence of 'non-collapsed tan mottled lungs' (OR 8.64, p>0.01). Hampshire was found as a protective factor with an (OR 0.138, p<0.01), while high percentage of Large white was found a risk factor, (OR 2.05, p=0.02). However, when adjusting for PCV2 antibodies in a bivariate logistic regression model only non-collapsed mottled lungs showed evidence of association (OR 11.63, p<0.01).

Correlation with outcome 'presence of PCV2 antibodies'

The presence of PCV2 antibodies was strongly associated with the genetic background. The presence of Meishan resulted in OR of 7.2 (p=0.1) and an increase of 25% of Landrace resulted in significantly lower odds to have PCV2 antibodies (OR 0.21, p=0.2). Moreover a negative correlation with a regression coefficient of -0.1 (95% CI -0.03 – -0.001) was found, indicating lowers titres of PCV2 antibodies with the increase in percentage of Landrace. Of post-mortem findings 'paleness of the kidney' was associated with the presence of PCV2 antibodies (OR 0.16, p=0.04).

DISCUSSION & OUTLOOK

Frequencies of lesions found in PCV2 PCR+ animals were similar to those describe by *Segales et al.* in 2004 in PMWS confirmed animals. Interpretation of results is hampered as no healthy animals were included in the post-mortem and therefore strength of association with PCV2 virus titres could only be compared between low and severely wasting animals. Small differences between these group would have required a larger sample size.

The inclusion of histopathological findings is needed to further investigate the use of PCV2 virus titre to predict the severity of presented clinical signs.

The most interesting finding of this study is the associations between PCV2 Elisa and percentage of breeds present in the sampled pigs. Results suggest a deficiency in the production of appropriate levels of antibodies in pigs with high Landrace %; the contrary was found for pigs with Meishan. These and the association of breeds with wasting are consistent with other studies that suggested a higher PMWS susceptibility of Landrace and higher resistance of Hampshire (*Opriessnig et al , 2009;* Bergström et al, 2006). However, calculation of breed percentages in this study are approximations based on breeds of grand-parents and parents. To further investigate the impact of breeds and to corroborate findings more details on breeding lines need to be sought.

Future work will also focus on the development of overall post-mortem score which might be more appropriate to investigate correlation with PCV2 virus titres. The overall score will be based on a weighted mathematical equation that integrates the findings of each of the post-mortem component analysed.

References:
- Bergström et al. *The incidence of thin-to-wasting pigs with respect to race in a progeny test station affected by PMWS.* Proc Int Pig Vet Soc, 2006, Vol 1, 169.
- Oppriessnig et al. *Difference in severity of porcine circovirus type 2 (PCV2)-induced pathological lesions between Landrace and Pietrain pigs.* J Anim Sci Journa, 2009. Online version (http://jas.fass.org).
- Segales, J., et al., *Pathological findings associated with naturally acquired porcine circovirus type 2 associated disease..* Vet Microbiol, 2004. **98**(2): p. 151-8.

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