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The relationship between porcine circovirus 2 antigen score and antibody titre and histology of lymph nodes in 375 euthanased sick and healthy pigs from 113 British pig farms with and without postweaning multisystemic wasting syndrome

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ABSTRACT

Data from a cross-sectional study of 113 British pig herds carried out in 2004 were used to investigate the associations between postweaning multisystemic wasting (PMWS) in pigs and herds and porcine circovirus 2 (PCV2) antigen score and antibody titre, and associated histological signs in lymph nodes. The sensitivity and specificity of published herd definitions for PMWS were tested on the study farms to consider the role of PCV2 in PMWS. Herds were defined as PMWS-affected, -unaffected or -recovered based on current and past postweaning mortality (PWM), grower pigs with clinical signs of rapid wasting, hairiness and pallor and no other known cause of death on the farm. PCV2 antigen and antibody were not used in the definition of PMWS. In each PMWS-affected herd, up to three sick pigs with the clinical signs above and one healthy pig of a similar age were taken for postmortem examination (PME). In all other herds at least one healthy pig was taken for PME. Lymph nodes were analysed for PCV2 antigen and histological changes, and serum samples were analysed for PCV2 antibody.

PCV2 antibody was present in all the herds sampled. There was a non-linear association between PCV2 antigen and antibody. There was no association between the presence of high scores of PCV2 antigen in pigs and the presence of high PWM in herds. PCV2 antigen score was significantly higher in sick than healthy pigs within farms, and high PCV2 score was associated with giant cells, coalescence and absence of germinal centres in lymph nodes. These results did not vary by PMWS-affected, -unaffected or -recovered farms. PCV2 antigen was present at high scores in approximately 10% of healthy pigs on all farms.

All three herd definitions of PMWS were highly sensitive, defining PMWS-affected herds as affected, but had a specificity ranging from 23% to 43%.

We conclude that the current diagnostic tests for PCV2 indicated higher scores of virus in sick pigs but were not useful to define pigs or herds with PMWS. The ubiquity of PCV2 and the lack of specificity of the PCV2 tests indicate that PCV2 may be a necessary but not sufficient cause of PMWS disease. Linking this with the knowledge that the herd breakdowns occurred in a space time epidemic indicates that another infectious co-factor may be necessary for disease to occur.
1. Introduction

Postweaning multisystemic wasting syndrome (PMWS) was first reported in Canada in 1991, in a high health pig herd (Harding, 2004). The disease is characterised by wasting, palor and occasional jaundice in nursery and fattening pigs (Segales and Domingo, 2002). Morbidity and mortality were 5–30% and close to 100%, respectively (Albina et al., 2001). PMWS is now a pig disease of global importance (Allan et al., 2004). PMWS was first reported in Great Britain (GB) in 1999. PMWS has been reproduced by direct contact of healthy pigs with PMWS affected pigs (Albina et al., 2001), indicating that it is an infectious disease. Analyses of patterns of spread of PMWS between farms within Denmark and within GB indicate that it is a new disease, most likely caused by a single infectious agent (Vigre et al., 2005; Woodbine et al., 2007).

Porcine circovirus 2 (PCV2) was isolated from pigs in Canada with PMWS (Ellis et al., 1998), and was attributed as the aetiological agent causing PMWS (Albina et al., 2001; Krakowka et al., 2001a; Fenaux et al., 2002; Allan et al., 2004). There is now evidence that PCV2 was circulating in pig populations free from PMWS well before PMWS was first reported (Sandvik et al., 2001) and in countries free from PMWS (Pensaert et al., 2001). There is no experiment that consistently fits Koch’s postulate that PCV2 demonstrably causes PMWS disease in pigs (Krakowka et al., 2000; Harms et al., 2001; Fenaux et al., 2002; Pensaert et al., 2004). The most reproducible disease occurs when non-infectious immunostimulants or co-infections (e.g. porcine respiratory and reproductive syndrome virus) are given to experimental piglets together with PCV2 (Allan et al., 2000, 2003; Magar et al., 2000; Botner et al., 2001; Krakowka et al., 2000, 2001a,b; Rovira et al., 2002). Recent research has proposed that only certain genetic variants of PCV2 may cause disease (Opiessnig et al., 2006), but again the evidence for this is not strong.

For every new and emerging infectious disease there is a period of time when the case definition for disease is redefined, e.g. bovine spongiform encephalitis (BSE) (Miller, 1999), acquired immunodeficiency syndrome (AIDS) (Cooley and Hamill, 1995), and severe acute respiratory syndrome (SARS) (Anon, 2003). The case definition for an infectious disease should apply to individual hosts rather than a population and ideally include a sufficient and necessary cause. The case definition for PMWS is still unresolved. All definitions to date define affected herds, rather than pigs, and include the presence or score of PCV2 antigen. There are six definitions for herd-level PMWS. Sorden (2000) uses enlarged lymph nodes, progressive dyspnoea, diarrhoea, pallor, jaundice and wasting, depletion of lymphoid tissue and high scores of PCV2 virus in a group of up to six pigs. Additions to this definition include a history of raised mortality on affected farms (Rose et al., 2003; Segales et al., 2005; pcvd.org), pigs with characteristic but non-specific signs of sudden wasting and pallor and no other known aetiological agent involved (Woodbine et al., 2007) and farm to farm spread of PMWS (consultative committee for emergency animal diseases Anon, 2005). To date there is no pig definition of PMWS.

In this paper we present the patterns of association between PCV2 antibody and antigen and histology of pigs collected from the herds investigated by Woodbine et al. (2007) and compare three herd definitions of PMWS with the definition used for the herds and pigs in the current study and evaluate the role of PCV2 in the definition of PMWS.

2. Materials and methods

2.1. Farm selection

Data on PCV2 antibody titre and antigen score and histological presentation of three lymph nodes were collected from 375 sick and healthy euthanased pigs taken from 113 herds in GB. These 113 herds were a subsample of 116 herds (because euthanased pigs were not taken from all herds visited). One hundred of these herds were from 500 randomly selected farmers that were contacted by post and who agreed to participate in the study. Their data were held by the British Quality Pigs database that contains virtually all herds with more than 100 breeding sows in England and Wales. Four herds were non-randomly selected from farms in England or Wales that were PMWS negative and 12 were non-randomly selected from farms in Scotland where farmers agreed to participate in the study. The geographical distribution, mean herd size and percent outdoor farms of randomly selected farms were representative of pig farms in GB using DEFRA data on herd size and location (Woodbine et al., 2007).

At the time of the cross-sectional study 85/116 herds were PMWS-affected, 44% (37) of these had been confirmed as PMWS positive by a laboratory (clinical signs, no other known cause and PCV2 antigen) and a further 51% (43) by the farmer’s veterinarian (clinical signs, no other known cause and raised mortality). Most of the farms not laboratory confirmed were the outbreaks of PMWS that started during the 2001 foot-and-mouth disease (FMD) epidemic when routine diagnostic procedures were halted (Woodbine et al., 2007). Ten of the 116 farmers considered that their farms had previously had the clinical signs mentioned above but were recovered (PMWS-recovered). Nineteen farmers reported that their herd had never raised postweaning mortality and pigs with the clinical signs, pallor and wasting (PMWS-unaffected), and 2 herds had unknown PMWS-status.

2.2. Mortality data

All PMWS-affected herds had raised postweaning mortality, which had not been attributed to any other disease by the farmer, veterinarian or laboratory. The current mortality (when the farm visit took place), and where applicable, peak mortality (highest on-farm mortality whilst being affected by PMWS) and pre-PMWS mortality (mortality on-farm before PMWS) figures for 6–16-week-old pigs were collected from each farm during the farm visit. Each farmer was interviewed on management practices, husbandry and clinical PMWS. Closed questions on PMWS mortality were also asked and are used in this paper. Answers regarding mortality rates were
validated where possible using farm records whilst on the farm and in a follow-up telephone questionnaire to all farmers.

2.3. Selected pigs and further examinations

Abnormal clinical signs observed in postweaning pigs on all the farms visited were recorded by the researchers. These included classic signs of PMWS (wasting, pallor and hairiness), and porcine dermatitis and nephropathy syndrome (PDNS) (blood encrusted skin mottling over the hind quarters and legs). On farms with clinical signs of PMWS the farmer and a trained member of the research team (all members who selected pigs were agricultural or science graduates with experience of working with pigs) identified and agreed on the age group of pigs most affected and up to three sick pigs, together with one healthy pig of the same age, were selected, euthanased and taken for postmortem examination (PME). Due to financial constraints only one healthy and up to three sick pigs were taken from each farm. Sick pigs were defined as those with the above-mentioned clinical signs. Where the number of sick pigs on the farm exceeded three, selection was based on taking a selection of pigs at different stages of disease. On the 19 PMWS-negative farms up to four pigs were selected for PME. A total of 375 pigs were taken from 113 herds.

Euthanased pigs were transported at the end of each farm visit to either Leeds Veterinary Laboratory (LVL) (all farms in England and Wales) or Scottish Agricultural College (SAC), Aberdeen (all farms in Scotland). All pigs were coded anonymously by farm and identity so that all PME were completed blind.

The following day a full PME was carried out by a qualified pathologist using a standard protocol designed for this project. The left or right tracheobronchial, ileocaecolic and inguinal lymph nodes were collected from each pig. Half of each lymph node was taken for histology and the other half transported to Queen’s University, Belfast for immunohistochemistry, where the PCV2 antigen score of each lymph node was determined as described by Krakowka et al. (2005). The quantity of viral antigen was scored between 0 and 4 based on the amount of PCV2 nucleocapsid staining. A negative result, 0 = no staining observed, +/− = possibly some virus, a score of 1+ = scattering of stained histiocytes and macrophages with no parenchymal cell involvement, 2+ = both single and focal antigen presence in the histiocytes and macrophages with rare parenchymal involvement, 3+ = multiple foci of affected histiocytes and syncytii, and affected follicular dendritic cells with occasional parenchymal involvement, and 4+ = extensive focal or confluent antigen affected cells and substantial antigen in parenchymal cells. The other half of each lymph node was read histologically at LVL using a standard form.

2.4. Blood samples

A sample of blood was collected immediately after death from pigs. Serum was removed from the whole blood and stored at −20 °C at the University of Warwick, England. Batches of serum were then sent to Queen’s University, Belfast where they were tested for PCV2 antibody. The PCV2 antibody titre was determined using an indirect peroxidase monolayer assay (IPMA) with fivefold dilutions.

2.5. Data analysis

Data were analysed in Excel, SAS and S plus. The statistical association between histological signs, PCV2 scores and PMWS-positive and -negative pigs were investigated. Significance was set at P ≤ 0.05 and the null hypothesis was that there was no association between a histological change/PCV2 score and the health of a pig. Chi squared or Fisher’s exact tests and Mann–Whitney tests were used to investigate associations ignoring herd. A rank test (Fowler et al., 1998), a non-parametric method for testing the within-farm equality of the distribution of antigen score between sick and healthy pigs was used. Farms with data from healthy and sick pigs (n = 94) were included in the analysis and a farm was signed 1 if it met the criteria and 0 if it did not meet the criteria. Six scenarios were tested; these were all sick pigs with a PCV2 score of ≥4+, ≥2+, or ≥1+ and at least one sick pig with these PCV2 scores. The farm signs for these six situations were then compared.

A receiver operator curve (ROC) was developed to identify the optimal cut off for PCV2 antigen score by health status of pigs for the current study. This cut off was used to investigate the associations between presence/absence of histological lesions and PCV2 antigen score >2+ or <3+ and PMWS-status of pigs and herds using previously proposed definitions for PMWS.

Finally, to investigate whether other herd level definitions of PMWS were sensitive or specific to define PMWS in the pigs or herds in the current study we estimated the sensitivity and specificity (Dahoo et al., 2003) of the four definitions for PMWS provided by Sorden (2000), Rose et al. (2003), Australia (Anon, 2005) and the presence of PCV2 >2+ in a pig or herd in the current study with the definition of PMWS in the current study (Woodbine et al., 2007) as the gold standard.

3. Results

The mean and median mortality on PMWS-affected and -recovered herds before the epidemic were 3.5% and 2.5% (range, 0.5–15.9%) and 3.4% and 3.3% (range, 1.0–10.5%), respectively. The mean and median mortality at the time of the visit on PMWS-unaffected farms were 3.2% and 3.0% (range, 0.5–15.9%) and 3.4% and 3.3% (range, 1.0–10.5%), respectively. The mean and median peak mortality on PMWS-affected and -recovered farms, were 19.1% and 17.91, 5 d.f., P < 0.05). Pigs with higher or lower PCV2 antigen scores were significantly more likely to have a lower serum PCV2 antibody titre than pigs with middle values for PCV2 antigen (Fig. 2) (Kruskal–Wallis K value of 17.91, 5 d.f., P < 0.05).
PCV2 antigen score was higher in sick pigs than healthy pigs within farms when adjusted for between farm variability using the sign test (Table 1). It was also higher irrespective of our classification of PMWS herd status (Table 2), indicating that there was more PCV2 antigen in sick pigs but that this did not correlate with the PMWS-status of the herd. The majority (90%) of healthy pigs had low PCV2 antigen score (<3+) but approximately 10% of healthy pigs from each type of PMWS herd had a PCV2 antigen score of >2+ (Table 2). There was no association between farm status and the distribution of PCV2 antigen among sick and healthy pigs (Table 2).

The optimal PCV2 antigen score to differentiate sick and healthy pigs was >2+ from the ROC curve. A PCV2 antigen score >2+ was statistically associated with coalescence in the ileocaecocolic lymph node, lymphoid depletion in the ileocaecocolic lymph node and giant cells and absence of germinal centres in all three lymph nodes (Table 3). These histological signs were also associated with sick pigs compared with healthy pigs (unpublished data).

When the current study definition (Table 4) of PMWS affected herds was used as the gold standard, the sensitivity and specificity of this cut off was 0.57 (95% CI, 0.49–0.65) and 0.19 (95% CI, 0.11–0.27). The sensitivity and specificity of the Sorden (2000) and Rose et al. (2003) or the Australian definition (Anon, 2005) (Table 4) were 0.90 (95% CI, 0.82–0.98) and 0.22 (95% CI, 0.14–0.29) and 1.00 (95% CI, 0.92–1.00) and 0.43 (95% CI, 0.35–0.51), respectively.

4. Discussion

Considerable thought was put into the definition of PMWS affected pigs and herds for this study. Raised postweaning mortality from an unknown cause was included in the definition of PMWS as it was likely to be reliably recalled because of the impact of PMWS on farm profitability and staff morale. In 2004, PMWS was the one syndrome causing unexplained raised postweaning

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**Table 1**

| Definition               | n farms | n sick pigs > PCV2 levels than healthy | % Non-tied Farms | Sign test result         |
|--------------------------|---------|----------------------------------------|------------------|--------------------------|
| At least 1 sick pig >4+ | 41      | 51                                     | 80.4            | Greater than expected by chance \( P < 0.01 \) |
| At least 1 sick pig >2+ | 58      | 66                                     | 87.9            | Greater than expected by chance \( P < 0.01 \) |
| At least 1 sick pig >1+ | 51      | 57                                     | 89.5            | Greater than expected by chance \( P < 0.01 \) |
| All sick pigs >4+       | –2      | 8                                      | 25.0            | No significant difference \( P = 0.11 \) |
| All sick pigs >2+       | 7       | 41                                     | 17.1            | Less than expected by chance \( P < 0.01 \) |
| All sick pigs >1+       | 12      | 46                                     | 26.1            | Less than expected by chance \( P < 0.01 \) |
mortality, so it seems likely that PMWS was the main cause of mortality on these farms. In addition, all but one farmer involved their veterinarian in investigations of raised postweaning mortality, suggesting that it was a real effect. The selection of sick pigs with signs of wasting, pallor and hairiness was used because these are the key clinical signs of PMWS, and were easy to recognise. The selection of healthy pigs from PMWS-affected and -unaffected farms gave two types of control (within and between herd classification of PMWS) that enabled us to investigate PCV2 diagnostic tests in sick and healthy pigs from both PMWS-affected and -unaffected herds. The detection of PCV2 antigen and antibody were deliberately omitted from the study definition of PMWS because of the confusing evidence from published challenge experiments. The definition of affected herds is supported by an analysis of geographical position and time of breakdown, which indicated a space-time clustered epidemic likely to be caused by a single pathogen (Woodbine et al., 2007).

The PCV2 antigen scores were significantly higher in sick pigs compared with healthy pigs and PCV2 virus scores were positively associated with abnormal histology of lymph nodes, suggesting a link between PCV2 and disease. However, when the PCV2 antigen scores were investigated as a diagnostic indicator for PMWS, it was highly sensitive but non-specific, and was therefore not useful to define PMWS-affected herds or pigs. This was also true for the other herd case definitions for PMWS that were tested, because PCV2 is used in all earlier case definitions of PMWS (Sorden, 2000; Rose et al., 2003).

Table 2
Number and percent of healthy and of one randomly selected sick pig with PCV2 antigen score >2+ or <3+ by PMWS farm status in Great Britain 2003–2004.

| Pig status                | Farm status                  | PMWS-affected | PMWS-recovered | PMWS-unaffected |
|---------------------------|------------------------------|---------------|----------------|-----------------|
| Healthy pigs              |                              | 9.8           | 13             | 18              |
| % PCV2 3+ or 4+           |                              | 90            | 88             | 82              |
| % PCV2-negative to 2+     |                              | 82            | 8              | 19              |
| No. of healthy pigs       |                              |               |                |                 |
| Randomly selected sick pig|                              | 43            | 60             | 44              |
| % PCV2 3+ or 4+           |                              | 57            | 40             | 56              |
| % PCV2-negative to 2+     |                              | 5             |                |                 |
| No. of sick pigs          |                              |               |                |                 |

* a PMWS-affected—raised mortality and current clinical signs.
  b PMWS-recovered—raised mortality but no current clinical signs.
  c PMWS-unaffected—no raised mortality or clinical signs.
  d 10 farms with no signs of raised mortality had no sick pigs.

Table 3
Associations between PCV2 antigen score (>2+ versus <3+) and histopathology 2003–2004.

| Lesion type* | Crude odds ratio | 95% CI Lower | Upper | Present in pigs examined (%) | Present in pigs with PCV2 > 2+ (%) |
|--------------|------------------|--------------|-------|-------------------------------|-----------------------------------|
| Coalescing IC| 2.4              | 1.4          | 4.2   | 17                            | 13                                |
| Multilocal follicles IC| 0.4 | 0.3          | 0.8   | 85                            | 77                                |
| Germinal centres TB   | 0.2              | 0.1          | 0.4   | 92                            | 83                                |
| Germinal centres IC   | 0.2              | 0.1          | 0.5   | 94                            | 86                                |
| Germinal centres ING  | 0.3              | 0.1          | 0.8   | 94                            | 88                                |
| Lymphoid depletion IC | 4.1              | 2.2          | 7.6   | 16                            | 40                                |
| Giant cells TB        | 2.3              | 1.1          | 4.9   | 8                             | 13                                |
| Giant cells IC        | 2.5              | 1.2          | 5.4   | 8                             | 13                                |
| Giant cells ING       | 2.8              | 1.3          | 6.3   | 7                             | 13                                |

* IC: ileocaecocolic, TB: tracheobronchial and ING: inguinal lymph node.

Table 4
The requirement for the presence of factors used/proposed to define a herd with postweaning multisystemic wasting by other authors (necessary = must be present in the herd for PMWS to be present).

| Definition of PMWS      | Clinical signs | Histological signs | Presence of PCV2 | Increased mortality in pigs 6–16 weeks old |
|-------------------------|----------------|--------------------|------------------|--------------------------------------------|
| Sorden (2000)           | Necessary      | Necessary          | Necessary        | Not necessary                              |
| Rose et al. (2003)      | Necessary      | Necessary          | Necessary        | Necessary                                  |
| Anon (2005)             | Necessary      | Necessary          | Necessary, PCV2 > 2+ | Necessary                                |
| Woodbine et al. (2007)  | Necessary      | Not necessary      | Not necessary    | Necessary                                  |
individuals. Herd level causality is a combination of individual processes plus the population level processes which define the similarity between individuals. In the case of PMWS, it has been easier to define excess postweaning mortality in herds and there are no unique clinical signs in individual pigs, and thus a herd level case definition has arisen.

We have demonstrated that the herd definitions that exist are not accurate for defining herds with PMWS. Inclusion of PCV2 antigen in the case definition greatly reduces the specificity of the diagnostic tests. In addition, an individual-based case definition (i.e. the actual pigs that are affected) remains elusive. This needs refining so that we can accurately define pigs and consequently herds and countries with PMWS. Consequently, we cannot currently (in 2008) accurately define the PMWS-status of individual pigs or herds. We have an incomplete case definition based on postweaning mortality, wasting and pallor in affected pigs and absence of other known pathogens.

There are several explanations for the association between PCV2 and all sick pigs. It might be possible that all the sick pigs in this study had PMWS. This would indicate that PMWS does not always cause raised postweaning mortality. Alternatively, there might have been misclassification of herds and those with PMWS were randomly distributed between herds with and without raised postweaning mortality or it might be that none of the herds had PMWS whatever their postweaning mortality levels. If any of the above are true then PCV2 does not cause high PWM and another agent, or co-factor, is causing the mortality known as PMWS.

Another hypothesis is that PCV2 is the causal agent, but that the current PCV2 diagnostics are not useful or our cross-sectional data were insufficient to demonstrate this. This might arise if PMWS occurs only when PCV2 infection occurs in particular age ranges, or via a certain route of entry, or only overwhelms certain pigs (Fig. 1) but by the time of disease all sick pigs and all herds have similar virus and antibody status. Finally, the current tests may be inadequately specific if they detect all PCV2 strains but only certain strains of PCV2 cause disease (Opriessnig et al., 2006).

The suggestion of viral overload comes from the pattern of association between PCV2 antigen and antibody (Fig. 1). When the PCV2 antigen score was low the PCV2 antibody titre was also low, indicating that either the virus level was low and being contained successfully or that there was a new infection and the antibody titres had yet to rise. At mid antigen scores there were high antibody titres, possibly containing the virus. However, at high antigen scores the antibody titres were low, indicating insufficient free antibody to contain the virus and that the immune system was overwhelmed. This is similar to that reported in human immunodeficiency virus (Pantophlet et al., 2004) where antigen overwhelms the antibody response.

In summary, if PCV2 is the causal agent of epidemic PMWS, and not just a virus that multiplies in all sick pigs, then we are far from understanding the pathogenic processes of this virus. If PCV2 is not the single causal agent of epidemic PMWS then we have a global epidemic pig disease still with unknown aetiology. The presence of PCV2, even in countries without epidemic PMWS (Jestin et al., 2001; Pensaert et al., 2001; Harding, 2004), and the lack of a robust experimental model with PCV2 alone indicate that presence of >2+ scores of PCV2 antigen as measured with the current test, is not a specific indicator for PMWS in individual pigs, and therefore cannot be used to define PMWS affected herds.

5. Conclusions

This study indicates that including PCV2 antigen scores in the case definition of PMWS in pigs and herds is highly sensitive but not-specific, and therefore not useful. We have an incomplete herd case definition based on postweaning mortality, wasting and pallor in affected pigs and absence of other known pathogens. We cannot accurately define individual pigs with PMWS. We conclude that the evidence from this paper combined with that of Woodbine et al. (2007) indicates that either a different infectious agent is causing PMWS or that the current diagnostic PCV2 antigen and antibody tests are not sensitive nor specific tools to define pigs with PMWS.

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