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Transmission of different variants of PCV2 and viral dynamics in a research facility with pigs mingled from PMWS-affected herds and non-affected herds

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ABSTRACT

Post-weaning Multisystemic Wasting Syndrome (PMWS) has been identified in most swine-producing countries worldwide. The disease has resulted in significant health challenges and economic damage to the swine industry. The aim of this study was to determine horizontal transmission of porcine circovirus type 2 (PCV2) and to examine viral dynamics in pigs in a controlled PMWS transmission study. In the study pigs from PMWS-affected herds and non-affected herds were permitted to have close contact (same pen), nose-to-nose contact (to pigs in neighbouring pens) or no physical contact (pen across the aisle and pens in other compartments). By DNA sequence analysis, eight variants of genotype PCV-2b were identified in the research facility. From the spread of these PCV2-variants it was concluded that PCV2 primarily infects through close contact and nose-to-nose contact. PCV2 genome sequences were obtained from selected pigs at arrival to the research facility and again when the same pigs developed PMWS. This analysis showed that pigs from PMWS-affected herds developed PMWS caused by the same variant of PCV2 as they carried when entering the research facility. In contrast, pigs from non-affected herds developed PMWS with PCV2-variants identified in pigs from PMWS-affected herds. This was probably connected to at least 10^3 higher mean serum-titer of PCV2 in pigs from PMWS-affected herds as compared to pigs from non-affected herds at the beginning of the transmission study. The study further showed that pigs able to control the PCV2 infection, as measured by the PCV2-titer in serum, recovered clinically (pigs from PMWS-affected herds) or stayed healthy (pigs from non-affected herds). Like this, pigs with a PCV2 titer below 5×10^8 copies/ml serum during the study period had a chance of recover from the PCV2 infection whereas pigs with PCV2 titers above 5×10^8 copies/ml serum at any time point generally died from PMWS.

1. Introduction

Post-weaning Multisystemic Wasting Syndrome (PMWS) was first observed in Canada in 1991 (Harding and Clark, 1997). It has today a worldwide distribution and is considered one of the most important swine diseases in Europe. Usually PMWS appears in pigs aged two to four months. Affected pigs show wasting or unthriftiness, enlarged lymph nodes, respiratory distress and occasionally jaundice and diarrhoea (Segalés et al., 2005a). The most distinct microscopic lesions in lymphoid organs are lymphoid cell depletion as well as formation of multinucleated giant cells and basophilic intracytoplasmic inclusion bodies (Segalés and Domingo, 2002). Infection with PCV2 is needed for full expression of PMWS. However, not all pigs infected with PCV2 will develop the disease (Segalés et al., 2005a).

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Infection with PCV2 is systemic (Bolin et al., 2001; Brunborg et al., 2004) with a higher viral load in serum and tissues of pigs suffering from PMWS as compared to healthy pigs (Brunborg et al., 2004; Liu et al., 2000; Olvera et al., 2004; Rovira et al., 2002; Segalés et al., 2005b). The respiratory system may be the route of entry, as suggested by the capacity of the virus to infect bronchial and bronchiolar epithelial cells (Magar et al., 2000). Alternatively or complementary, PCV2 may infect the nasopharynx and tonsils and spread via the blood or lymph (Magar et al., 2000). PCV2 has also been detected in bronchial, nasal, tonsillar, salivary, ocular, faecal and urinary swabs (Bolin et al., 2001; Krakowka et al., 2000; Segalés et al., 2005b; Shibata et al., 2003; Grau-Roma et al., 2008b), again with a significant higher viral load in secretions from PMWS-affected pigs (Segalés et al., 2005b; Grau-Roma et al., 2008b). This suggests that oro-nasal secretions as well as urine and faeces are potential routes of viral shedding.

The incubation period for development of PMWS is approximately 2 weeks (Albina et al., 2001). Interestingly, when PCV2-inoculated piglets were placed in contact with specific pathogen-free (SPF) piglets, disease in the contact-exposed SPF piglets was recorded 16 days after appearance of clinical signs in the inoculated pigs. This observation suggests that the contact-exposed SPF piglets became infected during the clinical phase of the inoculated piglets (Albina et al., 2001) where the viral load in secretions is expected to be high. PCV2 is able to persist in the pigs for a long period. Thus, infectious PCV2 viruses were detected in tissues of a single pig sacrificed at day 125 post-infection (Bolin et al., 2001). In blood and secretions, virus has been detected until post-infection day 70, the day of necropsy (Bolin et al., 2001). The long persistence of virus in secretions enables transmission of virus for a long period. Hence, direct contact with pigs carrying a 42-day-old PCV2 infection resulted in transmission of virus to 3 of 3 control pigs (Bolin et al., 2001).

PMWS commonly affects pigs 2–3 weeks after weaning (Harding and Clark, 1997) suggesting that natural infection occurs at about the time of weaning and mingling with other pigs. In order to establish disease control strategies, it is important to understand the horizontal transmission of PMWS and PCV2 in starter barns. In a transmission study, we have previously shown that pigs from PMWS-free herds developed PMWS following mingling with pigs with PMWS. PMWS developed in the PMWS-free pigs after close contact (same pen), nose-to-nose contact (to pigs in neighbouring pens) or no direct contact (pen across the aisle) (Kristensen et al., submitted for publication). In the present study, we specifically determined horizontal transmission of PCV2 and examined viral dynamics in pigs from this transmission study to elucidate if PCV2, in addition to PMWS, was transmitted from PMWS-affected pigs to non-affected pigs. This is important knowledge as it is unclear if transmission of the disease PMWS coincide with transmission of PCV2. Horizontal transmission of PCV2 was determined by studying the spread of different variants of PCV2 in the research facility, while viral dynamics was examined by quantifying the viral load in serum at different time points by real-time PCR.

2. Materials and methods

2.1. Experimental design and sampling

The PCV2 transmission study was performed with pigs collected from 6 different herds in Denmark as described elsewhere (Kristensen et al., submitted for publication; study II). Four of the herds showed problems with PMWS and had mortalities from 5 to 15%. All four herds were seropositive for PRRS (except herd E (Fig. 1)), PCV2 and mycoplasma (Kristensen et al., submitted for publication). Two of the herds were free from PMWS and had mortalities of approx. 2% (Kristensen et al., submitted for publication). These two herds were seropositive for PCV2 and mycoplasma (Kristensen et al., submitted for publication). From the four herds with PMWS, 27 pigs (6–11 weeks old) with clinical signs of PMWS (wasting and unthriftiness) were collected from each herd. From the two healthy herds, 144 pigs (4–5 weeks old) were collected from each herd. All the selected pigs from PMWS-affected herds (108 pigs) and 216 pigs from the non-affected herds were mingled in 4 compartments (Comp. 1–4) at the same farm (Fig. 1). As on-site controls, 18 of the selected pigs from the healthy herds were placed in a separate compartment (Comp. 5, Fig. 1). The pens in the compartments were separated by bars permitting the pigs to have nose-to-nose contact. As off-site controls, 54 of the selected pigs from the healthy herds were kept at a separate farm. The duration of the infection study was 46–48 days.

Blood samples were collected from all the pigs at arrival. In addition samples were collected 3 weeks later and at the termination of the study. The blood samples were centrifuged and serum stored at −80 °C. Clinical signs were recorded continuously during the study period. Pigs showing severe clinical signs referable to PMWS (wasting, unthriftiness, dyspnoea and diarrhoea) were euthanized

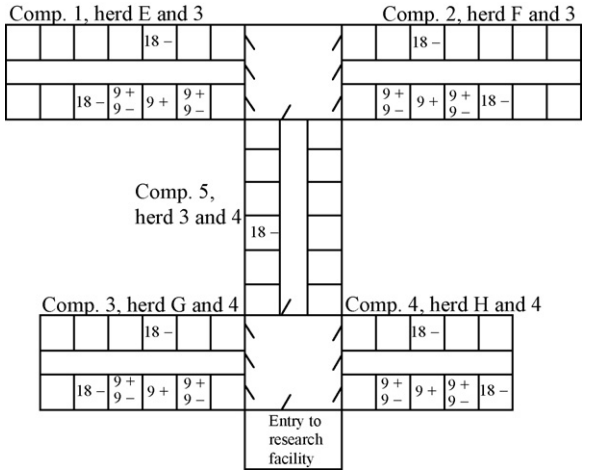

Fig. 1. The research facility used in the PCV2 transmission study. The pigs used in the study were collected from four PMWS-affected herds (herd E–H) and two non-affected herds (herd 3 and 4). Pigs from the PMWS-affected herds (+) were mingled with pigs from the non-affected herds (−) in pens as shown in the figure. The number of pigs in each pen is indicated. Bars, permitting the pigs to have nose-to-nose contact, separated the pens.