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Reproduction of post-weaning multi-systemic wasting syndrome in an animal disease model as a tool for vaccine testing under controlled conditions

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ABSTRACT

Snatch farrowed, colostrum deprived piglets were inoculated with different combinations of porcine circovirus 2, porcine parvovirus and *Erysipelothrix rhusiopathiae* candidate vaccines. 10 piglets were mock-vaccinated. Following virus challenge with a combined porcine circovirus 2/porcine parvovirus inoculum, all animals were monitored and samples taken for serology, immunohistochemistry and qPCR. At 24 dpc all non-vaccinated animals remaining were exhibiting signs of post-weaning multi-systemic wasting syndrome which was confirmed by laboratory analysis. Details of the study, analysis of samples and performance of the candidate vaccines are described.

1. Introduction

Porcine circovirus type 2 (PCV2) is a small, non-enveloped virus with a circular, single stranded DNA genome of approximately 1800 nucleotides (Meehan et al., 1998) belonging to the family *Circoviridae*, genus *Circovirus*. PCV2 is different antigenically and genomically from the non-pathogenic porcine circovirus type 1 (PCV1) (Tischer et al., 1982). The virus was first isolated and characterised in the late 1990s and has now been associated with a number of disease syndromes throughout the world including post-weaning multi-systemic wasting syndrome (PMWS) (Allan et al., 1998; Meehan et al., 1998). PCV2 has also been linked with a number of non-PMWS disorders including porcine respiratory disease complex (PRDC) and porcine dermatitis and nephropathy syndrome (PDNS) (Allan et al., 2000a).

PMWS most commonly affects pigs of 2–4 months of age, with mortality ranging from 4 to 20% (Segales and Domingo, 2002). The major clinical sign of PMWS is wasting in association with enlarged lymph nodes. Other signs include pallor of the skin, respiratory distress, and diarrhoea and icterus (Harding and Clark, 1997). PCV2 is now recognised as the causative agent of PMWS, the disease having been reproduced experimentally by the inoculation of pigs with PCV2 alone (Kennedy et al., 2000). However, PCV2 infected pigs do not always develop PMWS and it is thought that the development of full clinical disease will arise as a result of PCV2 infection in combination with other co-factors (Opriessnig and Halbur, 2012) such as presence of other pathogens (Allan et al., 1999), the age and source of pigs, environmental conditions, genetics of the pigs (Opriessnig et al., 2009) or non-infectious immunostimulation due to vaccination (Krakowka et al., 2001).

PMWS has had a major economic impact on the pig meat industry due to fewer pigs available for slaughter, poorer feed conversion rates and the costs of management of sick pigs, not only due to PMWS but also the secondary infections associated with immunosuppression (Segales et al., 2004). In England it has been estimated that a death from PMWS can cost a farmer £84.10. Prior to vaccination the annual cost to the English pig industry was estimated to be £52.6 million and £88 million during the epidemic years (Alarcon et al., 2013). In the United States, the disease has cost producers an average of 3–4 dollars per pig with peak losses up to 20 dollars per pig (Gillespie et al., 2009). PMWS is considered to have cost the European Union between €562 million and €900 million per year (Segales et al., 2006) during peak infection years.

The global swine industry increasingly relies on vaccination. With a total market value of €525 million in 2008, vaccines have become the most important health management tool for modern pig production (Vetnosis Ltd., March 2009).

However, it is important to establish not only the safety but also the efficacy of new PCV2 vaccines, especially those that may be formatted with other viral and/or bacterial antigens. Field studies tend to be very

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Table 1
Experimental sampling schedule for clotted bloods, faeces samples and necropsies.

Age (days)	Days post challenge (dpc)	Clotted bloods	Faeces	No. of necropsies/group			
				Group 1	Group 2	Group 3	Group 4
14	−12 (vaccination)	X					
28	−7 (booster)	X					
35	0 (challenge)	X					
42	7	X	X	3	2	2	3
45	10	X		2	3	3	2
52	17	X					
59/60	24/25	X	X	5	5	5	5[a]

[a] Group 4 animals were euthanized at 24 dpc.

expensive, can suffer from the lack of a discrete control group and often have many variables. The changeable nature of the external environment also means that field experiments are rarely replicable making conclusions tenuous. As such these experiments must be preceded with preliminary experimental studies. In this laboratory a successful disease model has been developed for the reproduction of PMWS which has been used to date for several PCV2 vaccine trials. This communication describes the use of this animal disease model as a means of vaccine testing and its potential role in the investigation and prevention of PCV2 associated diseases (PCVADs). The model is suitable for small scale testing of novel vaccine formulae and also for testing of other important biologicals such as the efficacy of antimicrobial feed additives.

2. Materials and methods

2.1. Experimental animals

Colostrum deprived piglets were obtained from a high health status, minimal disease breeder–finisher unit. Sows had been vaccinated against PPV and *Erysipelas*. Pigs were snatch farrowed and quickly transferred to a clean, previously fumigated, isolation house with an ambient temperature of 30 °C and bedded on a heat mat covered with clean wood shavings. The house was maintained under negative pressure with HEPA filtered air. The animals were immediately fed with colostrum substitute (Provita calf colostrum concentrate, Provita Eurotech, U.K.) as per manufacturer's instructions for 24 h. This was then replaced with sow milk substitute (Faramate, Volac, U.K.) followed by a milk replacer at weaning (Milkywean, Trou International Nutrition, Netherlands). Any scour or dehydration was treated with Spectam Scourhalt and Lectade Plus.

2.2. Viruses

A cell culture isolate of PCV2 from pigs with wasting disease in the Republic of Ireland was used in this study. This virus was isolated by inoculation of a pooled tissue homogenate into L35 cells and characterised as PCV2 by reactivity with specific monoclonal antibodies and as PCV2b by genomic sequence analysis. The titre of the pool was $10^{6.5}$ $TCID_{50}$/ml and endogenous retrovirus present in the L35 cell line was removed by chloroform treatment prior to animal inoculation.

A cell culture isolate of PPV from pigs with PMWS in Canada was also used in this study. This virus was isolated in primary pig kidney cells and purified by limiting dilutions. The titre of the pool in primary pig kidney cells was $10^{7.5}$ $TCID_{50}$/ml.

2.3. Vaccines

Vaccines were supplied by Merial SAS. They consisted of Circovac (an inactivated vaccine against PCV2), Parvoruvax (an inactivated vaccine against *Erysipelothrix rhusiopathiae* serotype 2 and PPV) and two experimental vaccines designated as Classical and Advanced, both designed to protect against PCV2 and PPV. Phosphate buffered saline (PBS) was used as a negative control vaccine.

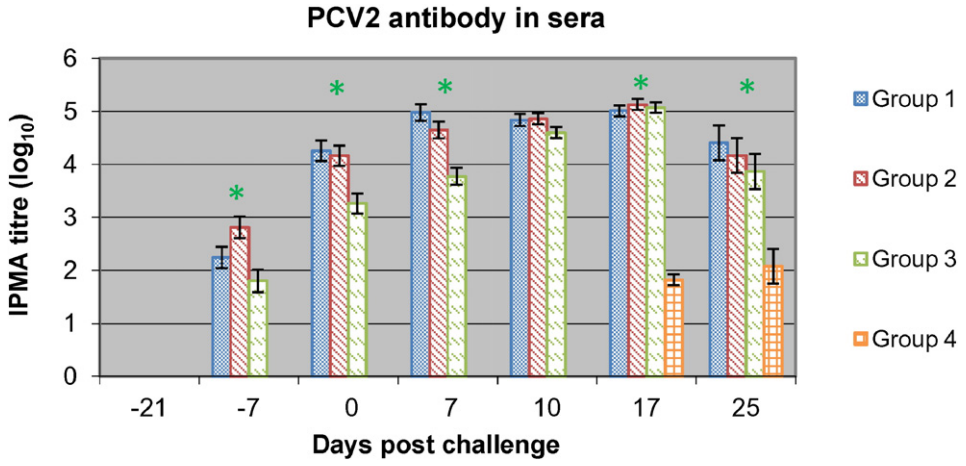

Fig. 1. PCV2 IPMA antibody levels in sera. Group 1 commercial vaccine combo (Circovac & Parvoruvax), Group 2 classical experimental vaccine (R901), Group 3 advanced experimental vaccine (V041 & W051), and Group 4 PBS controls. * denotes significant difference between means as determined by analysis of variance and Fisher's protected least significant difference (LSD) test. Error bars displayed are the standard error of the mean. Day −7, Group 2 is significantly higher than 3 ($P = 0.007$); day 0, Groups 1 and 2 are significantly higher than 3 ($P = 0.002$); day 7, Groups 1 and 2 are significantly higher than 3 ($P < 0.001$); day 17, Groups 1, 2 and 3 are significantly higher than 4 ($P < 0.001$); day 25, Groups 1, 2 and 3 are significantly higher than 4 ($P < 0.001$). There was no significant difference between groups on day 10 ($P > 0.05$). All groups were negative on day −21.