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EXPERIMENTALLY-INDUCED DISEASE

Experimental Reproduction of Porcine Circovirus Type 2 (PCV2)-Associated Enteritis in Pigs Infected with PCV2 Alone or Concurrently with *Lawsonia intracellularis* or *Salmonella typhimurium*

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Summary

Porcine circovirus (PCV)-associated disease (PCVAD) has emerged to become one of the most economically important pig diseases globally. One of the less commonly recognized clinical manifestations of PCVAD is PCV2 type 2 (PCV2)-associated enteritis in growing pigs; however, experimental confirmation of the ability of PCV2 alone or PCV2 coinfection with other agent(s) to induce enteritis is lacking. In this study, 120 specific-pathogen-free (SPF) pigs were divided randomly into six groups: controls (negative control pigs), PCV2 (inoculated with PCV2), LAW (inoculated with *Lawsonia intracellularis*), SALM (inoculated with *Salmonella typhimurium*), PCV2-LAW (concurrently inoculated with PCV2 and *Lawsonia intracellularis*) and PCV2-SALM (concurrently inoculated with PCV2 and *Salmonella typhimurium*). One half of the pigs in each group were subject to necropsy examination 14 days postinoculation (dpi) and the remaining pigs were examined at 28 dpi. The average daily weight gain was not different ($P > 0.05$) between groups. Individual pigs inoculated orally with PCV2 regardless of coinfection status (2/10 PCV2, 1/10 PCV2-LAW, 3/10 PCV2-SALM) developed PCVAD with diarrhoea and reduced weight gain or weight loss between 14 and 28 dpi. Those pigs had characteristic microscopic lesions in lymphoid and enteric tissues associated with abundant PCV2 antigen. Enteric lesions were characterized by necrosuppurative and proliferative enteritis with crypt elongation and epithelial hyperplasia in LAW and PCV2-LAW pigs by 14 dpi, ulcerative and necrosuppurative colitis in SALM and PCV2-SALM pigs by 14 dpi, and lymphohistiocytic enteritis with depletion of Peyer's patches in PCV2, PCV2-SALM and PCV2-LAW pigs by 28 dpi. To the authors' knowledge, this is the first report documenting that under experimental conditions, PCV2 can induce enteritis independently from other enteric pathogens and that oral challenge is a potentially important route and perhaps the natural route of PCV2 transmission in growing pigs.

Keywords: enteritis; *Lawsonia intracellularis*; porcine circovirus type 2 (PCV2); *Salmonella typhimurium*

Introduction

Porcine circovirus (PCV)-associated disease (PCVAD) can manifest clinically in a variety of ways in a pig or group of pigs (Allan and Ellis, 2000). One clinical manifestation of PCVAD reported under field conditions is diarrhoea; however, to the authors' knowledge, experimental reproduction of PCV type 2 (PCV2)-associated enteritis has not been described to date.

Field case reports indicate that granulomatous enteritis and diarrhoea can occur in animals infected

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with PCV2 and sometimes this is the only clinical sign of PCV2 infection (Segalés *et al.*, 2001; Chae, 2005). Although enteric disease is one of the less commonly recognized clinical manifestations of PCVAD, it has been shown that PCV2 can be detected readily in faeces and thus it has been proposed that the faecal–oral route is the main route of PCV2 transmission (Sato *et al.*, 2000; Yang *et al.*, 2003; Jensen *et al.*, 2006). PCV2 DNA can be detected by polymerase chain reaction (PCR) in faeces as early as 1 day postinoculation (dpi) and can still be detected at 70 dpi (Shibata *et al.*, 2003). When faecal samples from PCVAD and non-PCVAD affected pigs were tested by PCR, PCV2 DNA was found in 60% of animals under 1.5 months of age and 88.2% of animals older than 1.5 months (Segalés *et al.*, 2005). A threshold of 10^5 PCV2 DNA copies per ng of total DNA in faecal samples was proposed to differentiate between PCV2-affected and non-affected pigs (Segalés *et al.*, 2005). Furthermore, faecal shedding of PCV2 has been determined to be an appropriate indicator for PCV2 incidence and faecal shedding levels mimic levels shed by other routes in affected animals (Grau-Roma *et al.*, 2009).

PCV2-associated enteritis may occur any time in the grow-finish phase, can affect the small or large intestine and is commonly associated with the presence of other swine enteric pathogens, including *Salmonella typhimurium*, which when found together with PCV2 resulted in more severe diarrhoea of longer duration (Kim *et al.*, 2004; Jung *et al.*, 2006). Affected intestinal segments are sometimes thickened and necrotic, resembling gross lesions of *Lawsonia intracellularis* infection, and have been misdiagnosed as such (Jensen *et al.*, 2006). Microscopically, PCV2-associated enteritis has been described as having variable amounts of macrophages infiltrating the mucosa, with PCV2 antigen present in crypt epithelium and in macrophages in the lamina propria and submucosa. Villus atrophy and fusion together with multinucleated giant cells within the lamina propria have also been reported (Carrasco *et al.*, 2000; Núñez *et al.*, 2003; Kim *et al.*, 2004; Zlotowski *et al.*, 2008).

The aim of the present study was to develop a model for PCV2-associated enteritis and to determine if concurrent *S. typhimurium* or *L. intracellularis* infection with PCV2 increases the duration of faecal shedding of PCV2 and the severity of PCVAD and enteric lesions.

Materials and Methods

Animals and Housing

One-hundred-and-twenty conventional, specific-pathogen-free (SPF) pigs were purchased from a commercial source known to be free of PCV2, swine influenza virus (SIV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV) and swine hepatitis E virus (swine HEV) as determined by periodical serological monitoring (PCV2, SIV, PRRSV, PPV, swine HEV) and PCR on serum (PCV2, PRRSV, swine HEV). The pigs were weaned and brought to the research facility at 2 weeks of age. On arrival, the pigs were randomly into six groups and rooms with 20 pigs in each group. In each room the pigs were housed in five 1.2×1.5 m raised pens (four pigs per pen) with plastic slatted flooring. Each pen was equipped with a nipple water dispenser and a self-feeder. The pigs were fed a phase-based diet for growing pigs free of animal proteins and antibiotics (Nature's Match; Purina Mills, LLC, St Louis, Missouri).

Experimental Design

The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. The 120 pigs received a random number (using a computer random number generator), were grouped based on gender and weight and randomly divided into the following six groups ($n = 20$ pigs in each group): controls (negative control pigs), PCV2 (inoculated with PCV2), LAW (inoculated with *L. intracellularis*), SALM (inoculated with *S. typhimurium*), PCV2-LAW (co-inoculated with PCV2 and *L. intracellularis*) and PCV2-SALM (co-inoculated with PCV2 and *S. typhimurium*). Inoculation was done at 28 days of age. Half of the pigs in each group were subject to necropsy examination at 14 dpi and the remaining pigs were examined at 28 dpi. The selection of the pigs for necropsy examination at 14 or 28 dpi was done by using a random number generator. Serum samples and faecal samples were collected weekly. For collection of faecal samples, a sterile polyester-tipped swab (Fisherbrand®, Fisher Scientific International Inc., Pittsburgh, Pennsylvania) was passed into the rectum and approximately 2 g of faeces were collected from each pig.

Inoculation

PCV2. PCV2b isolate NC-16845 from a pig with PCVAD (GenBank accession number EU340258; Opriessnig *et al.*, 2008) was used for oral inoculation. All the pigs in groups PCV2, PCV2-LAW and PCV2-SALM received 5 ml of the infectious PCV2 stock at a dose of $10^{4.5}$ $TCID_{50}$ via gastric lavage using a size 14 French (Fr) rubber catheter (Sovereign™, Tyco/Healthcare, Mansfield, Massachusetts).

L. intracellularis. For inoculation, *L. intracellularis* isolate BI-110904 at a dose of 10^7 $TCID_{50}$ was used.