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Multiple routes of porcine circovirus type 2 transmission to piglets in the presence of maternal immunity

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ABSTRACT

Porcine circovirus 2 (PCV2), the cause of porcine circovirus-associated disease (PCVAD), is widespread in swine farms throughout the United States with vaccine controlling disease, but not eliminating infection. We examined the PCV2 virological and immunological status of sows, pre-suckling piglets, and the farrowing environment of sow farms to determine PCV2 exposure risks, transmission dynamics, and immunological impacts at the time of farrowing. PCV2 was widely distributed in animals and the farrowing environment of 6 midwestern US sow farms irrespective of sow vaccination status. High levels of PCV2 capsid-specific antibodies were observed in sow serum and colostrum and had no apparent effect on PCV2 transmission to and infection in piglets. In 281 pre-suckling piglets from 59 sows, PCV2 DNA was detected in 63% of serum samples and on 93% of axillary skin swabs. PCV2 was present in one or more samples from 58 of 59 sows and in the farrowing environment. Isolated infectious virus samples from sows, presuckling piglets, and the environment were shown by sequencing to be genetically similar from all farms. In conclusion, piglets are readily infected with PCV2 in utero and are under constant challenge by PCV2 through contact with infected sows and a contaminated farrowing environment. However, maternal immunity did not affect PCV2 transmission to piglets or the viral load in sows. These findings illustrate the importance of maternal infection, despite robust anti-PCV2 immunity, in early infection of newborn piglets, and the need to develop appropriate infection models for elucidation of mechanisms of protective immunity.

1. Introduction

Porcine circovirus 2 (PCV2) is widespread in swine farms throughout the United States and is the causative agent of PCV-associated disease (PCVAD) (Allan and Ellis, 2000; Puvanendiran et al., 2011). Vaccination of pigs, frequently around the time of weaning, is effective in preventing PCVAD and reducing the level of PCV2 in serum, but it does not eliminate infection (Fort et al., 2008; Opriessnig et al., 2008, 2010a). Since nearly all-finishing herds in the United States are vaccinated, a large-scale immunological selective pressure is being exerted on PCV2 for new strains that grow better in the presence of an anti-PCV2 vaccine response. A better understanding of the mechanisms of immunity against PCV2 is necessary in order to achieve virus elimination in addition to prevention of PCVAD.

Development of a relevant research model for elucidation of the mechanisms of anti-PCV2 immunity requires the knowledge of when and how pigs are exposed to and become productively infected with PCV2 in a natural farm setting. Infection in finishers appears to occur at 10–15 weeks of age, when they become viremic (Larochelle et al., 2003; Shibata et al., 2003) and nearly all finishing-age pigs are infected with PCV2 (Puvanendiran et al., 2011),

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suggesting that gilts and sows also are infected. Consistent with this likelihood, PCV2 DNA can be detected in breeding sows, newborn piglets, and piglets less than 10 weeks of age with no signs of disease (Grau-Roma et al., 2009; Patterson et al., 2011; Shen et al., 2010). Shedding of PCV2 occurs in colostrum, milk, feces, and in nasal and oral swabs (Fort et al., 2008; Gerber et al., 2011; Ha et al., 2009; Patterson et al., 2011; Shibata et al., 2003). PCV2 virus is also stable in the environment (Welch et al., 2006). All of this information suggests that piglets are exposed to PCV2 early in life, if not during gestation, as well as throughout the suckling period through contact with the sow and the environment.

Immune sows deliver anti-PCV2 antibodies to piglets via colostrum and milk (Gerber et al., 2011; Madson et al., 2009). Maternal antibodies are suggested to control, but not prevent, PCV2 infection in newborn piglets (Madson et al., 2009; McKeown et al., 2005). Vaccination of pregnant sows increases antibody levels, including neutralizing antibodies, in serum, colostrum and milk, but does not prevent infection in piglets (Gerber et al., 2011; Madson et al., 2009).

Here, we examined the PCV2 virological and immunological status of sows, pre-suckling piglets, and the farrowing environment of midwestern US sow farms to determine PCV2 exposure risks, transmission dynamics, and immunological impacts at the time of farrowing. A study of this size, depth, and vaccination status is novel. PCV2 was observed to be widely distributed in sow farms, present in sow serum and secretions even in the presence of high levels of antibodies, and present in pre-suckling piglet serum. Infectious PCV2 was recovered in cell culture and sequencing showed that multiple isolates were present. Piglet infection in utero was widespread, and piglets were under constant challenge of infection through contact with sows and the farrowing environment. Importantly, there was no difference in PCV2 transmission in herds practicing sow vaccination even though anti-PCV2 antibody levels were consistently elevated.

2. Materials and methods

2.1. On farm sampling

Six commercial sow farms from Minnesota, Iowa, and Indiana were enrolled, and 6–14 sows that farrowed on the same day were sampled at each farm for a total of fifty-nine sows and 281 piglets (Table 1). The farms were representative of typical commercial swine facilities, with a diverse spectrum of health status including farms that were PRRSv positive (Table 1, health status not shown) but were not practicing intentional exposure. PCV2 vaccination of sows on all farms occurred early in life, at weaning, according to common practice. A booster vaccination 3–4 weeks before farrowing with a one-dose product (Circumvent PCV, Intervet Inc., or Ingelvac CircoFLEX, Boehringer Ingelheim Vetmedica, Inc) was performed on two of the six farms (Table 1).

Up to 5 newborn piglets from each sow were bled pre-suckling in clot tubes. Piglets were snatched from the sow and moved to a clean area to prevent environmental contamination. Serum was isolated, aliquoted, and refrigerated (4 °C) for short-term storage. Colostrum was sampled from sows within 12 h after farrowing and the aqueous fraction was isolated. Sow feces was collected manually and placed in a Whirl-Pak bag (Nasco, Fort Atkinson, WI). A clean placenta sample was excised as soon as it was observed. All samples were stored frozen at −20 °C for later analysis. Oral swabs were taken from all sows by placing a sterile polyester tip applicator between cheek and gum for 5–10 s. Uterine fluid was collected using a deep cavity culture swab to minimize contamination by other fluids. Swab samples were taken for sow and piglet axillary skin surface and gestation crate floor and bar surfaces by swiping the area with a dry polyester tip applicator. Axillary skin surfaces were used since they are least likely to be directly exposed to environmental surfaces during and following farrowing, and were previously used to assess in utero viral exposure (personal communication, Dale Polson, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). Swabs were placed in 500 μl phosphate-buffered saline (PBS) and refrigerated for 12–24 h. The liquid was then spun down and frozen at −20 °C. An unused farrowing room was sampled before and after disinfection, using a swiffer-type pad to wipe along the concrete aisle, floor aisle, crate floor, crate bar, and crate walls. Each pad was mixed with 30 ml of PBS in a plastic zipper bag and the liquid was frozen at −20 °C. Approximately 3 days after farrowing, sows were bled in clot tubes as described, and sampling was repeated for sow feces, skin, and environmental surfaces (crate bars and floor). Stillborn fetuses and piglets that died within 3 days of farrowing (maximum 2 per sow) were swabbed on the axillary skin surface and tissue was harvested from thoracic fluid, inguinal lymph node (ILN), mesenteric lymph node (MLN), liver, lung, spleen, kidney, jejunum, ileum, colon, tonsil, and heart. Abdominal

Table 1
Farm information.

Attribute	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5	Farm 6
Size	3500 Sow	2400 Sow	1300 Sow	1700 Sow	2000 Sow	3200 Sow
Farm type	Farrow	Farrow to finish	Farrow to finish	Farrow	Farrow	Farrow
Parities	0–2	Mixed	Mixed	Mixed	Mixed	Mixed
Air handling	Filtered	Not filtered	Not filtered	Not filtered	Not filtered	Filtered
PRRS status	Negative	Positive	Negative	Stable[b]	Negative	Stable[b]
PCV2 sow vaccination	No	No	Yes[a]	No	Yes[a]	No
# sows sampled	14	10	9	11	9	6
# piglets sampled	67	50	45	46	43	30

[a] Pregnant sows were vaccinated 3–4 weeks pre-farrow with a one-dose product.
[b] PRRS virus stable farms are seropositive, but PCR negative.