Elimination of porcine respiratory coronavirus by early weaning and segregation

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Summary

Porcine respiratory coronavirus (PRCV) is considered a variant of transmissible gastroenteritis virus (TGEV). This virus is endemic in North America. Porcine respiratory coronavirus and TGEV may be differentiated on the basis of a blocking enzyme-linked immunosorbent assay. Negative status for PRCV is required by certain countries wishing to import swine from North America. A study was conducted to determine if PRCV-negative piglets could be produced from PRCV-positive sows by early weaning and removal off-site for rearing. Forty piglets were early weaned from a PRCV-positive sow herd and tested monthly for PRCV antibodies and virus for a total of 4 months. While some piglets tested positive for PRCV at the beginning of the study, all pigs tested negative at the end of the study. This study demonstrates a method by which PRCV-negative animals may be attained for the purposes of export to countries requiring PRCV-negative status.

Keywords: swine, porcine respiratory coronavirus, maternal antibody, early weaning

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Resumen - Eliminación del coronavirus respiratorio porcino mediante el destete temprano y la segregación

El coronavirus respiratorio porcino (PRCV por sus siglas en inglés) es considerado una variante del virus de la gastroenteritis transmisible (TGEV por sus siglas en inglés). Este virus es endémico en Norteamérica. El coronavirus respiratorio porcino y el TGEV pueden diferenciarse en base a un ensayo de unión enzimática inmunoabsorbente de bloqueo. El estatus negativo para el PRCV es requerido por ciertos países que desean importar cerdo de Norteamérica. Se condujo un estudio para determinar si se pudieran producir lechones negativos al PRCV nacidos de hembras PRCV positivas, al destetarlos de forma temprana y sacarlos a creer fuera de sitio. Se adelantó el destete de cuarenta lechones de un hato de hembras PRCV positivas y se les hicieron pruebas mensuales durante cuatro meses, en busca del virus y los anticuerpos contra PRCV. Aunque algunos lechones resultaron positivos al PRCV al inicio del estudio, todos los cerdos resultaron negativos al final del estudio. Este estudio demuestra un método por el cual se pueden obtener animales PRCV negativos para los propósitos de exportación a países que requieren el estatus negativo al PRCV.

Résumé - Élimination du coronavirus respiratoire porcin par sevrage précoce et ségrégation

Le coronavirus respiratoire porcin (VCRP) est considéré comme un variant du virus de la gastroentérite transmissible porcine (VGET). Ce virus est endémique en Amérique du Nord. Le VCRP et le VGET peuvent être différenciés par une épreuve immunoenzymatique bloquante. Un statut négatif pour le VCRP est requis par certains pays désirant importer des porcs de l'Amérique du Nord. Une étude a été menée afin de déterminer si des porcelets négatifs pour le VCRP pouvaient être obtenus de truies positives pour le VCRP en pratiquant un sevrage hâtif et en retirant les animaux pour les élever hors-site. Quarante porcelets furent sevrés hâtivement d'un troupeau de truies positives pour le VCRP et testés mensuellement pendant quatre mois pour des anticorps dirigés contre le VCRP de même que pour la présence du virus. Bien que certains porcelets se soient avérés positifs pour le VCRP au début de l'étude, tous les porcs se sont révélés négatifs à la fin de l'étude. Cette étude présente une méthode par laquelle des animaux négatifs pour le VCRP peuvent être obtenus pour fin d'exportation dans des pays qui demandent un statut négatif pour le VCRP.

Porcine respiratory coronavirus (PRCV) is considered a variant of the transmissible gastroenteritis virus (TGEV). Porcine respiratory coronavirus colonizes the respiratory tract of swine, as opposed to TGEV, which selectively infects and replicates in enterocytes in the small intestine.[1] There is limited to no shedding of PRCV from the intestinal tract. Porcine respiratory coronavirus is genetically and antigenically related to TGEV. Since the isolation of PRCV in 1984, and its widespread dissemination, the seroprevalence and clinical activity of TGEV has decreased.[1,2] Porcine respiratory coronavirus is endemic in North America. Pigs are infected by direct contact or airborne transmission. Swine population density, season, and swine-farm proximity influence the transmission and epidemiology of PRCV.[1]

Infections with PRCV are usually subclinical. Pigs may become infected after weaning, despite the presence of maternal antibodies. Primary exposure of sows to PRCV showed that only about 30% of sows produced IgA antibodies in milk.[3] Subsequent exposure increased the proportion of sows producing IgA to 84%. Porcine respiratory coronavirus and TGEV may be differentiated serologically by a commercial blocking enzyme-linked immunosorbent assay (ELISA).[3] Countries that have PRCV-negative status in their swine populations require PRCV-negative status on health certificates. Therefore, providing a method for PRCV elimination would allow positive North American herds to access markets that normally would be unavailable.

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A study was designed to investigate whether early weaning of pigs in a PRCV-positive herd could produce pigs negative for PRCV both by virus detection and antibody testing.

Animal welfare

Piglets were managed with due regard for their welfare. The source farm was a Canadian Quality Assurance certified farm. Piglets were housed in the receiving nursery according to the Recommended Code of Practice for Swine (National Farm Animal Care Council).[4]

Herd description

A 250-sow herd located in southwestern Ontario, Canada, was selected for the purpose of this study. This was a closed herd that had tested negative for porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae*, and TGEV and was positive for PRCV. Suitable gilts were selected as required from the finishing barn and brought back to the breeding area. Sows were farrowed on a weekly breeding schedule.

Materials and methods

Piglets were selected from this herd using a convenience sampling method and were weaned at approximately 7 days of age (range 5 to 12 days). The parity status of sows of selected litters was not recorded. Piglets were identified individually and transported to an off-site nursery that had been cleaned and disinfected. Upon arrival, the piglets were administered tulathromycin (Draxxin; Zoetis Animal Health, Kirkland, Quebec), 2.5 mg per kg, by intramuscular injection. Piglets were housed according to the Recommended Code of Practice.[4]

Piglets were placed in pens with slatted, coated flooring, with 10 piglets per pen. Each pen contained a heat lamp, and room temperature was held at 32°C for the first 2 weeks. Piglets were fed a milk supplement several times daily and were offered free choice creep feed. When piglets were 3 weeks of age, a commercial weaned-pig ration was gradually introduced. The first two weaning rations were medicated with chlortetracycline (Chlor-100; BioAgroMix, Mitchell, Ontario), 1 kg per tonne of feed, and tiamulin (Denagard; Novartis Animal Health, Mississauga, Ontario), 1.75 kg per tonne of feed.

Piglets were acclimatized to the nursery for 1 week. A baseline blood sample was obtained by jugular venipuncture at 2 weeks of age. Nasal swabs were obtained from each pig at this time using Dacron swabs (Becton Dickinson, Franklin Lakes, New Jersey). The serum samples were then couriered on ice to the Animal Health Laboratory (AHL), University of Guelph, Guelph, Ontario, and the nasal swabs were sent to the Diagnostic Laboratory, University of Montreal, Montreal, Quebec. Blood samples were then collected at monthly intervals for a total of four samples per pig. Nasal swabs were again collected at the last serologic sampling. Serum was tested for PRCV using a blocking ELISA at AHL. The nasal swabs were tested for PRCV by polymerase chain reaction (PCR) at the Diagnostic Laboratory, University of Montreal.

Blood samples and nasal swabs were collected from 40 piglets in June 2010 and tested as described. As all serum and nasal samples were negative for PRCV on this test, these piglets were removed from the study. Forty piglets were selected from 10 litters in July 2010 (Replicate 1) and protocols were followed as described. The study was repeated 6 months later (Replicate 2), with piglet selection late February of 2011. Forty piglets were selected from 13 litters and blood sampling commenced in March of 2011.

Strict biosecurity entry protocols were maintained, with a minimum downtime of 24 hours. A Danish entry system was observed in the nursery. This required leaving outdoor footwear on a mat in the office area and walking to a change room where coveralls were put on; a sink was available for hand washing. Barn boots were available only inside the barn, which was accessed via the change room. No additional pigs entered the nursery during the period of study.

Results

For the piglets selected in July 2010 (Replicate 1), results of testing serum samples and nasal swabs revealed 12 serum samples positive for PRCV by ELISA (Table 1). The 12 positive pigs were from four different litters. Nasal swabs were negative by PCR for all 40 animals.

In August 2010, only four of the 12 piglets that had been seropositive remained seropositive. By September 2010, all pigs tested were seronegative and continued to test negative in October. Test results were reported only for 32 piglets that remained to the end of the study (seven piglets had been sold for export and one had died). All nasal swabs were negative for PRCV by PCR at the end of Replicate 1.

In Replicate 2, forty piglets were again selected as described. Three animals from a single litter and a fourth piglet from a different liter tested positive for PRCV by blocking ELISA on the first blood sample (Table 2). All 40 animals were negative by PCR on nasal swabs. At the second test, one animal among the four originally seropositive piglets remained seropositive by ELISA (Table 2). Subsequently, all piglets were seronegative. Test results were recorded for the 23 piglets that remained to the end of the study. Seventeen piglets had been removed from this replicate: four had died from a *Streptococcus suis* infection and 13 had been sold as purebreds for export purposes. All nasal swabs were negative for PRCV by PCR at the end of Replicate 2.

Discussion

Porcine respiratory coronavirus and TGEV are species of coronavirus of the *Coronaviridae* family. These are enveloped viruses, and as such are stable when frozen, but somewhat labile at room temperature or higher. Porcine respiratory coronavirus is a deletion mutant of TGEV and infects respiratory epithelial cells, whereas TGEV infects villus epithelial cells of the small intestine. Porcine respiratory coronavirus is shed primarily in nasal secretions, but may be detected in feces due to limited tropism for intestinal cells.[1]

Swine density, season, and distance between farms influence the transmission patterns of PRCV. The virus is spread either by airborne transmission or through direct contact. Pigs become infected shortly after weaning, even in the presence of maternal antibodies. Maternal immunity persists to 8 to 16 weeks of age, depending on the concentration of antibody in colostrum at the time of parturition.[3] Susceptible pigs experimentally infected with PRCV shed the virus for less than 2 weeks.[1] Antibodies to PRCV in challenged pigs are detectable with a commercial blocking ELISA 42 to 48 weeks post challenge.[2]

Negative status for PRCV is an essential requirement for export of Canadian swine to certain countries. Because most swine herds in Ontario, Canada, are positive for PRCV, these herds may not export pigs to countries with the requirement for PRCV-negative status. This study demonstrates

that it is possible to early-wean piglets and produce pigs eligible for export to countries requiring a PRCV-negative status. However, producers wishing to use this methodology to produce PRCV-negative pigs should first determine that PRCV is not circulating in the farrowing room. This may be accomplished by taking nasal swabs from piglets for PCR testing for PRCV.

Previous work has described elimination of PRCV in a large wean-to-finish complex in Mexico that had become infected despite the negative status of the supplying sow herd.[5] The elimination protocol involved strict all-in, all-out measures accompanied by thorough cleaning and disinfecting of PRCV-infected barns. The sow herd continued to test negative to the virus, and thus piglets continued to be sourced from this herd for the wean-finish units. This is a labor-intensive endeavor. Because of the proximity of swine herds in Ontario, it is difficult to maintain a PRCV-negative sow status due to airborne transmission of this virus.

In this study, the sow herd (farrow-to-finish) was positive for PRCV. Unfortunately, the sows were not tested during the study. However, as this was an exporting herd (swine were being selected and sold worldwide), pigs in the finishing barn were regularly tested for swine pathogens, including PRCV. It is known that the finishing pigs were positive for PRCV, indicating virus exposure at some point earlier. A previous attempt had been made in 2009 to produce PRCV-negative piglets by early weaning and segregating to a separate nursery. The initial nursery population tested negative for PRCV after a month. However, piglets were added to the barn 6 weeks after the initial population had entered. Test results subsequent to this addition revealed that the animals were positive for PRCV and the project was terminated. The protocols were then modified to place piglets in the nursery on a single-fill basis. Biosecurity measures were improved in order to minimize transfer of the virus into the nursery, which included the use of a Danish entry system.

Maternal antibodies were not quantified in this study. Swine herds wishing to establish a similar health status in early-weaned piglets should confirm that there is no virus circulating in the farrowing room prior to initiating the project.

Implication

Under the conditions of this study, it is possible to produce PRCV-negative piglets from a PRCV-positive farrow-to-finish herd.

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Conflict of interest

None declared.

Disclaimer

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Table 1: Results of testing early-weaned piglets for porcine respiratory coronavirus by blocking enzyme-linked immunosorbent assay (Replicate 1)*

Animal ID†	10-Jul	10-Aug	10-Sep	10-Oct
a-1	Neg	Neg	Neg	Neg
a-2	Neg	Neg	Neg	Neg
a-3	Neg	Neg	Neg	NT
a-4	Neg	Neg	Neg	Neg
a-5	Neg	Neg	Neg	Neg
a-6	Neg	Neg	Neg	Neg
b-1	Neg	Neg	Neg	NT
b-2	Neg	Neg	Neg	Neg
b-3	Neg	Neg	Neg	Neg
b-4	Neg	Neg	Neg	NT
c-1	**Pos**	Neg	Neg	NT
c-2	Neg	Neg	Neg	Neg
c-3	Neg	Neg	Neg	Neg
c-4	Neg	Neg	Neg	NT
d-1	Neg	Neg	Neg	Neg
d-2	Neg	Neg	Neg	Neg
e-1	**Pos**	Neg	Neg	NT
e-2	**Pos**	**Pos**	Neg	Neg
e-3	**Pos**	Neg	Neg	Neg
f-1	**Pos**	Neg	Neg	Neg
g-1	**Pos**	**Pos**	Neg	Neg
g-2	**Pos**	**Pos**	Neg	Neg
g-3	**Pos**	**Pos**	Neg	Neg
h-1	Neg	Neg	Neg	Neg
h-2	Neg	Neg	Neg	Neg
h-3	Neg	Neg	Neg	Neg
i-1	**Pos**	Neg	Neg	Neg
i-2	**Pos**	Neg	Neg	Neg
i-3	**Pos**	Neg	Neg	Neg
j-1	Neg	Neg	Neg	Neg
j-2	Neg	Neg	Neg	Neg
j-3	Neg	Neg	Neg	NT
j-4	Neg	Neg	Neg	Neg

Table 1 continued

Animal ID†	10-Jul	10-Aug	10-Sep	10-Oct
k-1	Neg	Neg	Neg	Neg
k-2	Neg	Neg	Neg	NT
m-1	Neg	Neg	Neg	Neg
m-2	Neg	Neg	Neg	Neg
m-3	Neg	Neg	Neg	Neg
m-4	**Pos**	Neg	Neg	NT
m-5	Neg	Neg	Neg	Neg

* 40 piglets from 13 litters, early weaned at approximately 7 days of age (range 5-12 days), were treated with tulathromycin (Draxxin; Zoetis Animal Health, Kirkland, Quebec) at 2.5 mg/kg by intramuscular injection upon arrival at the nursery. Serum samples were taken every month and tested for PRCV (blocking ELISA, Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada). Polymerase chain reaction testing (Diagnostic Laboratory, University of Montreal, Montreal, Quebec) of nasal swabs for PRCV at onset and end of study were negative (results not shown).

† Each letter designates a litter; each number, a piglet in that litter.

NT = not tested (pig removed because of death or seedstock sale; one piglet died before testing began and is not included in the table).

the responsibility of the reader to use information responsibly and in accordance with the rules and regulations governing research or the practice of veterinary medicine in their country or region.

References

1. Saif LJ, Sestak K. Transmissible gastroenteritis and porcine respiratory coronavirus. In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ, eds. *Diseases of Swine.* 9th ed. Ames, Iowa: Iowa State University Press; 2006:489–516.

2. Constantini V, Lewis P, Alsop J, Templeton C, Saif LJ. Respiratory and fecal shedding of porcine respiratory coronavirus (PRCV) in sentinel weaned pigs and sequence of the partial S-gene of the PRCV isolates. *Arch Virol.* 2004;149:957–974.

3. Carman S, Josephson G, McEwen B, Maxie G, Antochi M, Eernisse K, Nayar G, Halbur P, Erickson G, Nilsson E. Field validation of a commercial blocking ELISA to differentiate antibody to transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus and to identify TGEV-infected swine herds. *J Vet Diagn Invest.* 2002;14:97–105.

4. National Farm Animal Care Council and Agriculture and Agri-Food Canada. Recommended code of practice for the care and handling of farm animals (Pigs). 1993. Communications Branch, Agriculture Canada, Ottawa, Ontario. Available at: **https://www.nfacc.ca/codes-of-practice/pigs**. Accessed 15 March 2015.

*5. Fano E, Torremorell M. Elimination of porcine respiratory coronavirus in a large wean-to-finish complex. *Proc IPVS*. Durban, South Africa. 2008:395.

*Non-refereed reference.

Table 2: Results of testing early-weaned piglets for porcine respiratory coronavirus by blocking enzyme-linked immunosorbent assay (Replicate 2)*

Animal ID†	7-Mar	28-Mar	2-May	20-Jun
a-1	Neg	Neg	Neg	NT
a-2	Neg	Neg	Neg	Neg
a-3	Neg	Neg	Neg	NT
b-1	Neg	NT	NT	NT
b-2	Neg	Neg	Neg	Neg
b-3	Neg	Neg	Neg	Neg
c-1	Neg	Neg	Neg	Neg
c-2	Neg	Neg	Neg	Neg
d-1	Neg	Neg	Neg	NT
d-2	Neg	Neg	Neg	Neg
d-3	Neg	Neg	Neg	Neg
d-4	Neg	Neg	Neg	NT
e-1	Neg	Neg	Neg	Neg
e-2	Neg	Neg	Neg	Neg
e-3	Neg	Neg	Neg	Neg
f-1	Neg	NT	NT	NT
f-2	Neg	Neg	Neg	NT
f-3	Neg	Neg	Neg	Neg
f-4	Neg	NT	NT	NT
f-5	Neg	Neg	Neg	Neg
g-1	Neg	Neg	Neg	Neg
g-2	Neg	Neg	Neg	Neg
h-1	Neg	Neg	Neg	Neg
h-2	Neg	Neg	Neg	NT
h-3	Neg	Neg	Neg	NT
i-1	**Pos**	Neg	Neg	NT
i-2	**Pos**	Neg	Neg	NT
i-3	**Pos**	Neg	Neg	Neg
j-1	**Pos**	**Pos**	Neg	Neg
j-2	Neg	Neg	Neg	Neg
j-3	Neg	Neg	Neg	Neg

Table 2 continued

Animal ID†	7-Mar	28-Mar	2-May	20-Jun
k-1	Neg	Neg	Neg	NT
k-2	Neg	Died	NT	NT
k-3	Neg	Neg	Neg	NT
k-4	Neg	NT	NT	NT
m-1	Neg	Neg	Neg	NT
m-2	Neg	Neg	Neg	Neg
m-3	Neg	Neg	Neg	Neg
m-4	Neg	Neg	Neg	Neg
n-1	Neg	Neg	Neg	Neg

* Litters, treatment, and testing described in Table 1.

† Each letter designates a litter; each number, a piglet in that litter.

NT = not tested (pig removed because of death or seedstock sale).