

PORCINE RESPIRATORY CORONAVIRUS



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SUMMARY

Etiology

- Porcine respiratory coronavirus (PRCV) is a single-stranded, negative-sense, RNA virus in the family *Coronaviridae*. It was first identified in Belgium in 1984. PRCV is a deletion mutant of the enteric coronavirus transmissible gastroenteritis virus (TGEV) and is also closely related to feline enteric coronavirus and canine coronavirus.
- Since it was first identified, various strains of PRCV have been described, many arising independently. Broadly, most strains are categorized as originating in the U.S. or Europe although Japanese strains have been described.

Cleaning and Disinfection

- Survival of PRCV in the environment is unclear. In PRCV endemic herds, virus can be isolated from pigs throughout the year. In other herds, PRCV temporarily disappears during summer months. PRCV may be highly stable when frozen, as is TGEV.
- Given the close relation of PRCV to TGEV, disinfection procedures may be extrapolated. TGEV is susceptible to iodides, quaternary ammonium compounds, phenols, phenol plus aldehyde, beta-propiolactone, ethylenamine, formalin, sodium hydroxide, and sodium hypochlorite. Alcohols and accelerated hydrogen peroxides reduce TGEV titers by 3 and 4 logs, respectively. A pH higher than 8.0 reduces the half-life of TGEV to 3.5 hours at 37°C (98.6°F) in cell culture. Like TGEV, PRCV may be inactivated by sunlight or ultraviolet light.

Epidemiology

- PRCV has been identified in Europe, the U.S., Canada, Croatia, Japan, and Korea. Current PRCV prevalence is unknown as PRCV is generally considered to cause mild disease and is most important for its potential to confound diagnosis of TGEV. Many asymptomatic herds may be identified by serology.
- PRCV is not zoonotic and infects only swine.

Transmission

- PRCV is spread via aerosol and direct contact between pigs. This usually occurs post-weaning when maternally derived antibody-mediated protection begins to decline. Transmission may also occur in growers/finishers when PRCV-naïve pigs are introduced.

- PRCV transmission is dependent on season in some herds, swine population density, the number of pigs on neighboring farms, and the proximity of neighboring swine farms. In highly swine-dense areas PRCV can spread several kilometers by aerosol.

Infection in Swine/Pathogenesis

- PRCV generally causes subclinical infection. When clinical respiratory disease is seen it is usually mild; however, severe cases have been described. Whether disease is subclinical or clinical, characteristic cranioventral consolidation of the lungs occurs. Other viral co-infections may increase the severity of respiratory disease.
- Although closely related to TGEV, most PRCV strains do not infect the gastrointestinal (GI) tract.

Diagnosis

- PRCV is diagnosed antemortem by isolating virus from nasal swabs. Swine testicular (ST) cells are most frequently used for virus isolation but other cell types have been described.
- Serological diagnosis of PRCV infection is frequently done using a blocking enzyme-linked immunosorbent assay (ELISA), which allows for the differentiation between PRCV and TGEV infection. This assay is available through the Iowa State University Veterinary Diagnostic Laboratory.
- PRCV infection can be diagnosed in the lungs postmortem using direct or indirect fluorescent antibody testing (FAT) and immunohistochemistry (IHC), in pigs with no enteric disease. FAT has also been described for identification of infected, though unidentified cells in the GI tract of experimentally infected animals. If enteric and respiratory disease are present concurrently, antigen detection techniques cannot differentiate PRCV and TGEV.
- PRCV nucleic acid may be detected in lung samples using *in situ* hybridization (ISH), cDNA probes, gene chip microarrays, reverse-transcription polymerase chain reaction (RT-PCR), nested RT-PCR, and quantitative RT-PCR (qPCR). Nucleic acid-based identification methods are typically the most sensitive and can distinguish between PRCV and TGEV.

Immunity

- A single vaccine for PRCV has been described. A recombinant adenovirus expressing the PRCV spike glycoprotein was found to be antigenic and partially protected vaccinated piglets upon PRCV challenge.
- PRCV has been investigated as a tool for vaccination against TGEV.
- Humoral immunity is not long lasting and pigs can be reinfected with PRCV.

Prevention and Control

- As PRCV infection generally causes subclinical or mild disease, to date there has been little to no effort toward prevention or control of infection.
- PRCV can be eliminated from herds by using an all-in all-out method where piglets are weaned early and transported to facilities that are PRCV-free. All pigs born to PRCV-positive sows would test positive for PRCV by serology early on, but later would test negative as maternal antibody wanes.
- Establishment of PRCV-negative herds is possible. Maintenance of PRCV-negative status can be achieved using strict biosecurity measures.

Gaps in Preparedness

- There are no PRCV vaccines that are commercially available.

OVERVIEW

Porcine respiratory coronavirus (PRCV) is a member of the family *Coronaviridae*, subfamily *Coronavirinae*, genus *Alphacoronavirus*, and species *Alphacoronavirus 1*. It is a deletion mutant of transmissible gastroenteritis virus (TGEV) and is closely related to feline enteric coronavirus and canine coronavirus. A single isolate arose in Belgium in 1984 and subsequently spread throughout Europe. At least seven independently arising PRCV isolates have been detected in the U.S. between 1980 and 2000. PRCV is also found in Japan, Korea, Croatia, and Slovenia, though other countries where pigs are raised and TGEV is present may also be positive for PRCV.

PRCV is known to exist only in swine. It is not zoonotic and does not have a non-swine reservoir. PRCV generally causes subclinical infection in swine, though some isolates have been associated with severe respiratory disease and experimental infection of pigs has resulted in severe respiratory disease and death. Virulence appears to be isolate specific. Circulation of PRCV occurs year-round in some herds and seasonally in others, disappearing during the summer months. Predictors of a herd becoming positive for PRCV include status of neighboring farms, proximity of neighboring farms, and swine density in the area. PRCV is efficiently spread via aerosol, up to several kilometers in areas of high swine density, and direct contact with infected pigs.

PRCV infection is generally asymptomatic. When disease is present pigs may exhibit, tachypnea, polypnea, dyspnea, sneezing, coughing, hyperthermia, anorexia, and delayed growth. Mortality is negligible. Pigs become infected within a few weeks after weaning in PRCV-endemic herds and when naïve pigs are comingled on nursery farms or introduced into endemic herds.

An indication of PRCV infection is seroconversion to TGEV with the absence of any clinical signs. PRCV-positive pigs cannot be distinguished serologically from TGEV using virus neutralization tests. Differentiation of PRCV vs. TGEV infection is done using the blocking/competitive inhibition enzyme linked immunosorbent assay (ELISA). Monitoring for PRCV infection in a herd may be accomplished by performing virus isolation from nasal swabs. Alternatively, the reverse transcription polymerase chain reaction (RT-PCR) assay can be used.

Post-mortem, PRCV antigen can be detected by using immunohistochemistry (IHC) and fluorescent antibody testing (FAT). Nucleic acid can be identified using *in situ* hybridization (ISH), microarray, cDNA probes, or RT-PCR. Techniques that detect nucleic acid can differentiate between PRCV and TGEV, while antigen detection techniques cannot. Appropriate samples for post-mortem testing include upper and lower respiratory tissues, tonsils, and nasal swabs. Ante-mortem samples include nasal swabs and serum.

A single vaccine has been described for PRCV. An adenovirus recombinant vector expressing the S protein in place of the adenovirus E3 gene was shown to be immunogenic in pigs. The vaccine was also shown to provide partial protection from PRCV infection, demonstrated by reduced nasal shedding of virus. No vaccines are currently available commercially. PRCV itself has been pursued as a vaccine candidate to prevent TGEV.

As PRCV is generally a subclinical disease, the overall threat it poses to herd health is unclear. However, PRCV-positive status of a herd may have economic implications as some countries will not import animals that are PRCV-positive. In order to address this, PRCV can be eliminated from pig populations through a process of early weaning and segregation followed by strict adherence to biosecurity procedures. Such measures may require concerted efforts among pig-rearing entities as PRCV is easily spread to nearby farms.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Porcine respiratory coronavirus (PRCV) is a member of the family *Coronaviridae*, subfamily *Coronavirinae*, genus *Alphacoronavirus*, and species *Alphacoronavirus 1*. PRCV is a single-stranded, positive-sense RNA virus that is pleomorphic, enveloped, and has large surface proteins that protrude from the membrane giving the virus the appearance of a crown.¹ PRCV is a deletion mutant of transmissible gastroenteritis virus (TGEV) and the two differ most importantly in the spike (S) glycoprotein. The deletion within S results in the absence of an antigenic site (termed site D), which allows for differentiation between PRCV and TGEV.^{2,3} PRCV is also closely related to feline enteric coronavirus and canine coronavirus.¹

1.2 Strain Variability

PRCV isolates generally fall into two categories, European or U.S., though other isolates have been described in Canada⁴, Japan, and Korea⁵. The European isolate arose in Belgium⁶ and then spread throughout Europe,¹ whereas the U.S. isolates (at least 7 have been described) appear to have arisen independently.⁷⁻¹⁰ Variability between strains occurs in the size of the deletion within the S gene (600–700 base pairs) as well as one of the subgenomic RNAs, open reading frame 3 and 3–1, which can be found during replication.^{5,9} Virulence in pigs varies with the isolate.¹¹

2. Cleaning and Disinfection

2.1 Survival

Survival of PRCV in the environment is unclear. In PRCV-endemic herds, virus can be isolated from pigs throughout the year. In other herds, PRCV temporarily disappears during summer months. PRCV may be highly stable when frozen, as is TGEV.¹

2.2 Disinfection

Given the close relation of PRCV to TGEV, disinfection procedures may be extrapolated. TGEV is susceptible to iodides, quaternary ammonium compounds, phenols, phenol plus aldehyde, beta-propiolactone, ethylenamine, formalin, sodium hydroxide, and sodium hypochlorite.¹ Alcohols and accelerated hydrogen peroxides reduce TGEV titers by 3 and 4 logs, respectively.^{12,13} A pH higher than 8.0 reduces the half-life of TGEV to 3.5 hours at 37°C (98.6°F) in cell culture.¹⁴ Like TGEV, PRCV may be inactivated by sunlight or ultraviolet light.¹

3. Epidemiology

3.1 Species Affected

PRCV has been described only in swine.

3.2 Zoonotic Potential

PRCV is not a zoonotic virus and poses no risk to humans.¹

3.3 Geographic Distribution

PRCV was first isolated in 1984 in Belgium⁶ and has since become endemic in Europe with additional reports from Denmark, France, Spain, and the United Kingdom (UK).¹ There have been at least seven separate PRCVs identified in the U.S.⁷⁻¹⁰ A limited serological survey in Iowa suggested that TGEV-free herds that became seropositive were actually exposed to PRCV.¹ PRCV isolates have also been identified

in Japan¹⁵, Korea^{16,17}, Slovenia¹⁸, and Croatia.¹⁹ Presence of PRCV in other countries has not been described.

3.4 Morbidity and mortality

PRCV is generally a subclinical infection though mild respiratory signs may occur in some herds and severe disease has been described in experimental pigs²⁰, depending on the isolate.²¹ Mortality has rarely been described as a direct result of PRCV infection.²⁰

4. Transmission

PRCV is transmitted via aerosol and direct contact between pigs.²² Virus was recovered from experimentally infected pigs in air samples for 6 days after infection. The highest quantities of virus were recovered days 2 and 4 post-infection. Concurrently collected nasal swab samples were positive for PRCV for 8 days post-infection (DPI).²³

5. Infection in Swine/Pathogenesis

PRCV replicates in the upper and lower respiratory tract (alveolar cells, nasal mucosa, tracheal, bronchial, and bronchiolar epithelium, alveolar macrophage, and tonsils²²) and can be isolated readily from nasal swabs for 6–10 days post-infection.^{24,25} Replication in the intestine has been shown by some to occur in a few, unidentified cells located underneath the epithelial layer.^{22,26} Virus titers peak between 3–5 DPI in the nasal mucosa, trachea, lung, tonsil, and bronchial lymph nodes.^{22,27} This is confirmed by studies using a fluorescent antibody test (FAT) showing peak fluorescence between 3–5 DPI.²⁸ Virus titer peak appears to depend on the age of the pigs with younger pigs having higher titer peak than older.²⁷ Viremia may occur as early as 2 DPI and may explain the isolation of virus from mesenteric lymph nodes and intestinal tissues that has been described by some investigators.¹⁰ Experimental data support a role for PRCV in exacerbation of clinical signs in co-infected animals.²⁹⁻³¹

5.1 Clinical Signs

PRCV infection is generally subclinical but reported clinical signs include polypnea, dyspnea^{4,32}, tachypnea³², sneezing, coughing^{32,33}, fever³², anorexia³², and delayed growth.³⁴ Severe respiratory distress and death are rarely described.²⁰ Clinical signs occur between 4–10 DPI in experimentally infected pigs and symptoms resolve by 14 DPI.³²

5.2 Postmortem Lesions

Gross PRCV lesions include multifocal to coalescing areas of consolidation, which may involve parts of all lobes. Interlobular edema may be present. Gross lesions are the most severe at 10 DPI and resolve by 15 DPI.³⁵

Microscopic lesions include thickening of interstitial septa, lymphoplasmacytic and histiocytic bronchiolar and alveolar exudate, type II pneumocytes hypertrophy and proliferation, airway epithelial necrosis, squamous metaplasia, dysplasia, and proliferation in all sizes of airway. Similar to the gross lesions, pulmonary lesions peak in severity at 10 DPI and resolve by 15 DPI.³⁵ The histopathological diagnosis is necrotizing and proliferative bronchointerstitial pneumonia.³⁵

6. Diagnosis

6.1 Clinical History

Nursery age pigs (1–2 weeks post-weaning³⁶) with high TGEV titers and mild to moderate respiratory disease should be suspected of having PRCV.³⁵ Most frequently PRCV is identified in herds with a history of seroconversion to TGEV in the absence of the typical clinical signs of TGEV (high mortality in

piglets and diarrhea in all ages of pigs).⁶ PRCV has also been isolated from TGEV outbreaks of reduced virulence¹⁰ and severe endemic respiratory disease in nursery pigs.³²

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

PRCV is easily isolated from nasal swabs or lung tissue following inoculation onto ST cells³⁷ or other swine origin cultured cells (PD5³⁷ or primary porcine kidney cells²). Confirmation of PRCV infection can be achieved by performing virus neutralization (VN) to TGEV¹⁵ if the original isolate came from the respiratory tract and there was no evidence of enteric disease. Alternatively, immunofluorescence (IF) can be performed on the cultured cells.³⁸ VN and IF cannot distinguish between PRCV and TGEV. Therefore, if a sample could be TGEV rather than PRCV (i.e. the animal exhibited signs of enteric disease), molecular techniques such as reverse transcription polymerase chain reaction (RT-PCR), *in situ* hybridization (ISH), microarray, or cDNA probes should be used to distinguish between the two viruses.

Detection of PRCV nucleic acid can be performed using RT-PCR (conventional⁸ or quantitative²⁴, nested³⁹, and in combination with restriction fragment length polymorphism⁴⁰).

- Conventional RT-PCR requires PCR product resolution on a gel and identifying mobility differences between the S gene of the sample and a TGEV positive control⁸. Following amplification, the PCR products may be subjected to restriction endonuclease digestion followed by fragment resolution on an agarose gel. These fragments can subsequently be sequenced as well as being compared to positive controls.⁴⁰ This technique is very labor intensive and may not be suitable for producing results rapidly.
- Nested RT-PCR amplifies a region of interest within the S gene in the first round of amplification. In a second round of amplification, a region within the first PCR product is generated. The size difference between a TGEV sample and a PRCV sample is indicative of the size of the deletion in the S gene in the PRCV genome. Using this technique on nasal swabs, PRCV genomic RNA could be detected for 5 or 6 days longer than blocking enzyme linked immunosorbent assay (ELISA) or IF on inoculated cultured cells.³⁹
- qRT-PCR can be used to identify PRCV in a sequence-specific manner in one step. Two probes are used, one that binds to the N gene and one that binds to the S gene. The S gene probe is specific for part of the gene that has been deleted in PRCV. A PRCV positive sample will only give a positive signal for the N gene probe, while a TGEV positive sample will give a positive signal for both the N and S genes.²⁴

ISH⁴¹, microarray^{42,43}, or cDNA probes^{44,45} can also be used to specifically identify PRCV viral nucleic acid.

- ISH involves the use of radioactive probes⁴¹ or non-radioactive digoxigenin-labeled probes⁴⁶, one of which reacts to both PRCV and TGEV, the other reacts only to TGEV. ISH can be used on inoculated cultured cells or in tissue sections to identify PRCV.⁴¹
- Microarrays can be used to specifically identify PRCV following viral RNA extraction, reverse transcription, cDNA amplification and labeling, and hybridization with the microarray. Unique sequences of PRCV-specific cDNA that are complementary to the amplified sequences are bound by the labeled cDNA and the binding can be visualized using an array scanner.^{42,43}
- cDNA probe technology requires that cultured cells be inoculated with virus. Cells are ideally inoculated at a low multiplicity of infection in order to achieve a one-step growth curve and less than 24 hours later, cell lysates are collected and spotted onto nylon membranes. The radioactively labeled or immunochemiluminescently labeled cDNA probes are then hybridized to the nylon membrane. Two sets of probes are used to distinguish between TGEV and PRCV.^{44,45}

IHC⁴¹ and FAT^{47,48} can be used to identify PRCV-infected respiratory tissues.⁴¹ In clinical samples taken from the intestine, a second method to confirm exposure to PRCV rather than TGEV is necessary because

use of antigen testing methods on tissue are not PRCV-specific.²⁸ This could be achieved using the blocking ELISA that is able to distinguish PRCV from TGEV infection in serum samples.² In samples that are inoculated onto cultured cells, IF can be used to identify PRCV.³⁸ The sensitivity of these assays has been shown to be lower than RT-PCR.^{39,47}

A double antibody-sandwich ELISA has been described to detect PRCV antigen. Three monoclonal antibodies (mAb), 25C9 (recognizes antigenic site A of S), 44C11 (recognizes antigenic site D of S), and 25H7 (recognizes the N protein) are coated onto plates.^{48,49} This assay cannot distinguish between PRCV and TGEV.

6.3 Tests to Detect Antibody

A blocking/competitive inhibition antibody ELISA has been described that can differentiate TGEV from PRCV.² The mAb that is used in the assay recognizes TGEV but not PRCV.⁴⁸ The ELISA has been standardized and made commercially available for use outside of the U.S., but is not licensed by the U.S. Department of Agriculture (USDA).^{5,15} The blocking ELISA is available through the Iowa State University College of Veterinary Medicine Veterinary Diagnostic Laboratory⁵⁰, among others. The blocking ELISA is the test of choice when it is important that animals be negative for TGEV/PRCV for export.⁴⁸ All other antibody detection ELISAs cannot differentiate between TGEV and PRCV.

VN tests can be used to determine TGEV/PRCV neutralizing antibody titers by utilizing a plaque reduction test.⁵¹ This assay cannot distinguish between TGEV and PRCV. VN tests can be used as a screening tool with subsequent utilization of the blocking ELISA to distinguish between TGEV and PRCV.¹⁵

6.4 Samples

Nasal swabs are the preferred antemortem sample for virus isolation^{7,36}, RT-PCR⁴⁹, and IF⁵¹. Both upper and lower respiratory tract tissues are appropriate postmortem samples for virus isolation³⁶, IF, IHC⁴¹, ISH⁴¹, RT-PCR, and FAT.^{36,43} Serum is the most appropriate ante- or postmortem sample for identification of PRCV antibody⁴⁸ and can also be used for virus isolation²⁸ or RT-PCR, potentially, to look for viremia. Biosecurity protocols for necropsy should be followed to prevent contamination of the environment.

7. Immunity

7.1 Post-exposure

Serum neutralizing antibodies can be detected beginning around 6 DPI upon primary infection with PRCV. The antibody response peaks approximately 14 DPI and subsequently wanes.²⁰ Following PRCV infection, neutralizing antibodies can be found in milk at minimal levels from 7–14 days post-farrowing with an increase in titers as lactation continues.⁵² Milk IgA levels vary among individuals following a single infection and reinfection with PRCV results in an increase in IgA detected in milk.⁵²

The duration of effective PRCV-induced immunity appears to be relatively short lived. PRCV-induced neutralizing antibody (nAb) titers are high at three weeks post-infection (WPI), low by 36 WPI, and marginal to absent one year post-infection.²⁵ Repeated infection with PRCV can be achieved. Additionally, within 1–2 weeks post-weaning, pigs become susceptible to PRCV infection as passive immunity wanes.⁵²⁻⁵⁴

7.2 Vaccines

A single PRCV vaccine has been described, which uses a recombinant adenovirus vector to express S in place of the adenovirus E3 gene.⁵⁵ The recombinant S was found to be immunogenic in pigs and partially protected vaccinated piglets from PRCV infection as measured by virus shedding in nasal swabs.⁵⁶ No

subsequent studies have been published using this vaccine and it was not determined whether the reduction in nasal shedding of virus was sufficient to prevent infection of uninfected sentinel animals placed in the same space.

After it was noted early on that incidence of TGEV infection appeared to decrease concomitantly with PRCV identification^{6,57}, interest began in understanding whether PRCV had a role in protection against TGEV. PRCV infection has been examined by multiple groups for its potential to protect pigs against TGEV disease. Partial protection against TGEV-induced mortality of anywhere between 11–67%^{54,58-62} has been found in piglets born to sows with prior exposure to PRCV. One laboratory found the lowest rates of mortality following TGEV challenge were in the second litter of piglets of multiparous sows that had been exposed to PRCV during two pregnancies.^{54,61}

7.3 Cross-protection

PRCV infection induces antibodies that cross-react with anti-TGEV antibodies. PRCV infection leads to the production of neutralizing antibodies to antigenic site A within the S protein but not the D site.^{37,55} This lack of D site reactivity is the factor that allows differentiation between PRCV and TGEV serologically.² It also may be that lack of site D reactivity is a reason that prior PRCV infection is not fully cross-protective against TGEV challenge.

8. Prevention and Control

PRCV is a deletion mutant of TGEV and non-swine reservoir species have not been described. Prevention of PRCV may require the prevention and control of TGEV. In Europe, as PRCV became endemic, incidence of TGEV outbreaks decreased.⁶ As PRCV is generally a subclinical infection, identification of positive herds will require regular testing. Monitoring for PRCV can be done by serology and is available at the Iowa State University College of Veterinary Medicine Veterinary Diagnostic Laboratory⁵⁰, among others.

Establishing and maintaining a PRCV-negative herd may be important economically as some export markets require swine to be PRCV-negative. It is possible to establish a PRCV-negative herd by early weaning of piglets from seropositive sows and removal to a clean facility.⁶³ Maintenance of a PRCV-negative facility may be achieved using strict biosecurity protocols. It is important to consider the PRCV status of neighboring herds, herd size, and proximity of neighboring herds as PRCV can spread several kilometers by aerosol.¹

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

PRCV is not addressed by the 2015 OIE Terrestrial Animal Health Code. No recommendations are in place on the importation of swine or pork with respect to PRCV.

10. Gaps in Preparedness

There are no commercially available PRCV vaccines available for use in pigs. While PRCV is not generally thought to cause serious respiratory diseases, a response plan to deal with an outbreak should be developed and further investigation into effective vaccines should occur.

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REFERENCES

1. Saif LJ, Pensaert MB, Sestak K, Yeo S, Jung K. Coronaviruses. In: Zimmerman JJ, Karkiker LA, Ramirez A, Schwartz KJ, Stevenson GW, eds. *Diseases of Swine*. 10 ed. Ames, IA: John Wiley & Sons Ltd; 2012:501-524.
2. Callebaut P, Pensaert MB, Hooyberghs J. A competitive inhibition ELISA for the differentiation of serum antibodies from pigs infected with transmissible gastroenteritis virus (TGEV) or with the TGEV-related porcine respiratory coronavirus. *Vet Microbiol*. 1989;20(1):9-19.
3. Rasschaert D, Duarte M, Laude H. Porcine respiratory coronavirus differs from transmissible gastroenteritis virus by a few genomic deletions. *J Gen Virol*. 1990;71 (Pt 11):2599-2607.
4. Jabrane A, Girard C, Elazhary Y. Pathogenicity of porcine respiratory coronavirus isolated in Québec. *Can Vet J*. 1994;35(2):86-92.
5. Usami Y, Fukai K, Ichikawa Y, et al. Virological and serological studies of porcine respiratory coronavirus infection on a Japanese farm. *J Vet Med Sci*. 2008;70(9):929-936.
6. Pensaert M, Callebaut P, Vergote J. Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis. *Vet Q*. 1986;8(3):257-261.
7. Wesley RD, Woods RD, Hill HT, Biwer JD. Evidence for a porcine respiratory coronavirus, antigenically similar to transmissible gastroenteritis virus, in the United States. *J Vet Diagn Invest*. 1990;2(4):312-317.
8. Vaughn EM, Halbur PG, Paul PS. Three new isolates of porcine respiratory coronavirus with various pathogenicities and spike (S) gene deletions. *J Clin Microbiol*. 1994;32(7):1809-1812.
9. Vaughn EM, Halbur PG, Paul PS. Sequence comparison of porcine respiratory coronavirus isolates reveals heterogeneity in the S, 3, and 3-1 genes. *J Virol*. 1995;69(5):3176-3184.
10. Kim L, Hayes J, Lewis P, Parwani AV, Chang KO, Saif LJ. Molecular characterization and pathogenesis of transmissible gastroenteritis coronavirus (TGEV) and porcine respiratory coronavirus (PRCV) field isolates co-circulating in a swine herd. *Arch Virol*. 2000;145(6):1133-1147.
11. Zhang X, Hasoksuz M, Spiro D, et al. Complete genomic sequences, a key residue in the spike protein and deletions in nonstructural protein 3b of US strains of the virulent and attenuated coronaviruses, transmissible gastroenteritis virus and porcine respiratory coronavirus. *Virology*. 2007;358(2):424-435.
12. Goyal SM, Chander Y, Yezli S, Otter JA. Evaluating the virucidal efficacy of hydrogen peroxide vapour. *J Hosp Infect*. 2014;86(4):255-259.
13. Hulkower RL, Casanova LM, Rutala WA, Weber DJ, Sobsey MD. Inactivation of surrogate coronaviruses on hard surfaces by health care germicides. *Am J Infect Control*. 2011;39(5):401-407.
14. Pocock DH, Garwes DJ. The influence of pH on the growth and stability of transmissible gastroenteritis virus in vitro. *Arch Virol*. 1975;49(2-3):239-247.
15. Miyazaki A, Fukuda M, Kuga K, Takagi M, Tsunemitsu H. Prevalence of antibodies against transmissible gastroenteritis virus and porcine respiratory coronavirus among pigs in six regions in Japan. *J Vet Med Sci*. 2010;72(7):943-946.
16. Chae C, Kim O, Min K, Choi C, Kim J, Cho W. Seroprevalence of porcine respiratory coronavirus in selected Korean pigs. *Prev Vet Med*. 2000;46(4):293-296.
17. Ahn K, Chae C, Kweon CH. Immunohistochemical identification of porcine respiratory coronavirus antigen in the lung of conventional pigs. *Vet Pathol*. 1997;34(2):167-169.
18. Vengust G, Valencak Z, Bidovec A. A serological survey of selected pathogens in wild boar in Slovenia. *J Vet Med B Infect Dis Vet Public Health*. 2006;53(1):24-27.
19. Roic B, Jemersic L, Terzic S, Keros T, Balatinec J, Florijancic T. Prevalence of antibodies to selected viral pathogens in wild boars (*Sus scrofa*) in Croatia in 2005-06 and 2009-10. *J Wildl Dis*. 2012;48(1):131-137.

20. van Nieuwstadt AP, Zetstra T, Boonstra J. Infection with porcine respiratory coronavirus does not fully protect pigs against intestinal transmissible gastroenteritis virus. *Vet Rec.* 1989;125(3):58-60.
21. Britton P, Mawditt KL, Page KW. The cloning and sequencing of the virion protein genes from a British isolate of porcine respiratory coronavirus: comparison with transmissible gastroenteritis virus genes. *Virus Res.* 1991;21(3):181-198.
22. Cox E, Hooyberghs J, Pensaert MB. Sites of replication of a porcine respiratory coronavirus related to transmissible gastroenteritis virus. *Res Vet Sci.* 1990;48(2):165-169.
23. Bourgueil E, Hutet E, Cariolet R, Vannier P. Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion. *Vet Microbiol.* 1992;31(1):11-18.
24. Hermann JR, Brockmeier SL, Yoon KJ, Zimmerman JJ. Detection of respiratory pathogens in air samples from acutely infected pigs. *Can J Vet Res.* 2008;72(4):367-370.
25. Wesley R. Neutralizing antibody decay and lack of contact transmission after inoculation of 3- and 4-day-old piglets with porcine respiratory coronavirus. *J Vet Diagn Invest.* 2002;14(6):525-527.
26. Cox E, Pensaert MB, Callebaut P, van Deun K. Intestinal replication of a porcine respiratory coronavirus closely related antigenically to the enteric transmissible gastroenteritis virus. *Vet Microbiol.* 1990;23(1-4):237-243.
27. Cox E, Pensaert M, Hooyberghs J, Van Deun K. Sites of replication of a porcine respiratory coronavirus in 5-week-old pigs with or without maternal antibodies. *Adv Exp Med Biol.* 1990;276:429-433.
28. Laude H, Van Reeth K, Pensaert M. Porcine respiratory coronavirus: molecular features and virus-host interactions. *Vet Res.* 1993;24(2):125-150.
29. Brockmeier SL, Loving CL, Nicholson TL, Palmer MV. Coinfection of pigs with porcine respiratory coronavirus and Bordetella bronchiseptica. *Vet Microbiol.* 2008;128(1-2):36-47.
30. Van Reeth K, Nauwynck H, Pensaert M. Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study. *Vet Microbiol.* 1996;48(3-4):325-335.
31. Lanza I, Brown IH, Paton DJ. Pathogenicity of concurrent infection of pigs with porcine respiratory coronavirus and swine influenza virus. *Res Vet Sci.* 1992;53(3):309-314.
32. Halbur PG, Pallarés FJ, Opriessnig T, Vaughn EM, Paul PS. Pathogenicity of three isolates of porcine respiratory coronavirus in the USA. *Vet Rec.* 2003;152(12):358-361.
33. Jung K, Renukaradhya GJ, Alekseev KP, Fang Y, Tang Y, Saif LJ. Porcine reproductive and respiratory syndrome virus modifies innate immunity and alters disease outcome in pigs subsequently infected with porcine respiratory coronavirus: implications for respiratory viral co-infections. *J Gen Virol.* 2009;90(Pt 11):2713-2723.
34. Vannier P. Disorders induced by the experimental infection of pigs with the porcine respiratory coronavirus (P.R.C.V.). *Zentralbl Veterinarmed B.* 1990;37(3):177-180.
35. Halbur PG, Paul PS, Vaughn EM, Andrews JJ. Experimental reproduction of pneumonia in gnotobiotic pigs with porcine respiratory coronavirus isolate AR310. *J Vet Diagn Invest.* 1993;5(2):184-188.
36. Janke BH. Diagnosis of viral respiratory disease in swine. *J Swine Health Prod.* 1995 3(3):116-120.
37. Callebaut P, Correa I, Pensaert M, Jiménez G, Enjuanes L. Antigenic differentiation between transmissible gastroenteritis virus of swine and a related porcine respiratory coronavirus. *J Gen Virol.* 1988;69 (Pt 7):1725-1730.
38. Cornaglia E, Chrétien N, Charara S, Elazhary Y. Detection of porcine respiratory coronavirus and transmissible gastroenteritis virus by an enzyme-linked immunosorbent assay. *Vet Microbiol.* 1994;42(4):349-359.
39. Kim L, Chang KO, Sestak K, Parwani A, Saif LJ. Development of a reverse transcription-nested polymerase chain reaction assay for differential diagnosis of transmissible gastroenteritis virus

- and porcine respiratory coronavirus from feces and nasal swabs of infected pigs. *J Vet Diagn Invest.* 2000;12(4):385-388.
40. Kwon HM, Saif LJ, Jackwood DJ. Field isolates of transmissible gastroenteritis virus differ at the molecular level from the Miller and Purdue virulent and attenuated strains and from porcine respiratory coronaviruses. *J Vet Med Sci.* 1998;60(5):589-597.
 41. Sirinarumitr T, Paul PS, Kluge JP, Halbur PG. In situ hybridization technique for the detection of swine enteric and respiratory coronaviruses, transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV), in formalin-fixed paraffin-embedded tissues. *J Virol Methods.* 1996;56(2):149-160.
 42. Chen Q, Li J, Deng Z, Xiong W, Wang Q, Hu YQ. Comprehensive detection and identification of seven animal coronaviruses and human respiratory coronavirus 229E with a microarray hybridization assay. *Intervirology.* 2010;53(2):95-104.
 43. Nicholson TL, Kukielka D, Vincent AL, Brockmeier SL, Miller LC, Faaberg KS. Utility of a panviral microarray for detection of swine respiratory viruses in clinical samples. *J Clin Microbiol.* 2011;49(4):1542-1548.
 44. Bae I, Jackwood DJ, Benfield DA, Saif LJ, Wesley RD, Hill H. Differentiation of transmissible gastroenteritis virus from porcine respiratory coronavirus and other antigenically related coronaviruses by using cDNA probes specific for the 5' region of the S glycoprotein gene. *J Clin Microbiol.* 1991;29(1):215-218.
 45. Vaughn EM, Halbur PG, Paul PS. Use of nonradioactive cDNA probes to differentiate porcine respiratory coronavirus and transmissible gastroenteritis virus isolates. *J Vet Diagn Invest.* 1996;8(2):241-244.
 46. Sirinarumitr T, Paul PS, Halbur PG, Kluge JP. An overview of immunological and genetic methods for detecting swine coronaviruses, transmissible gastroenteritis virus, and porcine respiratory coronavirus in tissues. *Adv Exp Med Biol.* 1997;412:37-46.
 47. Paton D, Ibata G, Sands J, McGoldrick A. Detection of transmissible gastroenteritis virus by RT-PCR and differentiation from porcine respiratory coronavirus. *J Virol Methods.* 1997;66(2):303-309.
 48. World Organization for Animal Health (OIE). Transmissible Gastroenteritis. *OIE Terrestrial Manual* 2008:1153-1163.
 49. Costantini 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(5):957-974.
 50. Iowa State University College of Veterinary Medicine Veterinary Diagnostic Laboratory. Diagnostic tests. <https://vetmed.iastate.edu/vdl/diagnostic-tests/>, 2016.
 51. Brim TA, VanCott JL, Lunney JK, Saif LJ. Cellular immune responses of pigs after primary inoculation with porcine respiratory coronavirus or transmissible gastroenteritis virus and challenge with transmissible gastroenteritis virus. *Vet Immunol Immunopathol.* 1995;48(1-2):35-54.
 52. Callebaut P, Cox E, Pensaert M, Van Deun K. Induction of milk IgA antibodies by porcine respiratory coronavirus infection. *Adv Exp Med Biol.* 1990;276:421-428.
 53. Saif LJ. Coronavirus immunogens. *Vet Microbiol.* 1993;37(3-4):285-297.
 54. Sestak K, Lanza I, Park SK, Weilnau PA, Saif LJ. Contribution of passive immunity to porcine respiratory coronavirus to protection against transmissible gastroenteritis virus challenge exposure in suckling pigs. *Am J Vet Res.* 1996;57(5):664-671.
 55. Callebaut P, Pensaert M. Expression and immunogenicity of the spike glycoprotein of porcine respiratory coronavirus encoded in the E3 region of adenovirus. *Adv Exp Med Biol.* 1995;380:265-270.
 56. Callebaut P, Enjuanes L, Pensaert M. An adenovirus recombinant expressing the spike glycoprotein of porcine respiratory coronavirus is immunogenic in swine. *J Gen Virol.* 1996;77 (Pt 2):309-313.

57. Saif LJ. Mucosal immunity: an overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease. *Vet Immunol Immunopathol.* 1996;54(1-4):163-169.
58. Bernard S, Bottreau E, Aynaud JM, Have P, Szymansky J. Natural infection with the porcine respiratory coronavirus induces protective lactogenic immunity against transmissible gastroenteritis. *Vet Microbiol.* 1989;21(1):1-8.
59. Paton DJ, Brown IH. Sows infected in pregnancy with porcine respiratory coronavirus show no evidence of protecting their sucking piglets against transmissible gastroenteritis. *Vet Res Commun.* 1990;14(4):329-337.
60. Saif LJ, van Cott JL, Brim TA. Immunity to transmissible gastroenteritis virus and porcine respiratory coronavirus infections in swine. *Vet Immunol Immunopathol.* 1994;43(1-3):89-97.
61. Lanza I, Shoup DI, Saif LJ. Lactogenic immunity and milk antibody isotypes to transmissible gastroenteritis virus in sows exposed to porcine respiratory coronavirus during pregnancy. *Am J Vet Res.* 1995;56(6):739-748.
62. Wesley RD, Lager KM. Increased litter survival rates, reduced clinical illness and better lactogenic immunity against TGEV in gilts that were primed as neonates with porcine respiratory coronavirus (PRCV). *Vet Microbiol.* 2003;95(3):175-186.
63. Burlatschenko S, Arsenault C. Elimination of porcine respiratory coronavirus by early weaning and segregation. *J Swine Health Prod.* 2015;4(23):208-213