PORCINE CYTOMEGALOVIRUS

Prepared for the Swine Health Information Center
By the Center for Food Security and Public Health,
College of Veterinary Medicine,
Iowa State University
December 2015

SUMMARY

Etiology

- Porcine cytomegalovirus (PCMV) is an enveloped DNA virus belonging to the family *Herpesviridae* and the subfamily *Betaherpesvirinae*.
- Infection with PCMV has previously been known as 'inclusion body rhinitis' based on the histopathological characteristics of the disease.

Cleaning and Disinfection

- Little is known about PCMV in the environment. The virus appears to survive in subzero temperatures.
- Chloroform and ether inactivate PCMV. Povidone-iodine (7.5%) is effective against human cytomegalovirus (human herpesvirus 5). Most herpesviruses are also susceptible to 30% ethanol and isopropanol, 1% sodium hypochlorite, formaldehyde, 0.12% ortho-phenylphenol, and 0.04% glutaraldehyde.

Epidemiology

- Swine are the natural host for PCMV.
- Infection with PCMV has not been documented in humans; however, concern about transmission through xenotransplantation exists.
- PCMV is endemic in nearly all swine populations worldwide, including North America, with seroprevalence approaching 100% in many areas.
- While neonates can develop fatal systemic disease, death is rare in older pigs. However, co-infections may increase morbidity and mortality when present.

Transmission

- PCMV is shed in nasal secretions, ocular secretions, urine, and cervical fluid. Transmission is primarily via direct contact, but congenital transmission also occurs.

Infection in Swine/Pathogenesis

- Clinical signs are rare except in neonates, where shivering, sneezing, respiratory distress, poor weight gain, and rhinitis have been observed, as well as conjunctival discharge and black discoloration around the eyes. Neurological signs can also occur.

- In older pigs, PCMV has been associated with porcine respiratory disease complex (PRDC). Reproductive losses can occur in pregnant sows that become infected.
- Latent PCMV infections can develop and become reactivated when pigs are stressed

Diagnosis

- Traditionally, PCMV is diagnosed via histological examination of tissue sections (staining, *in situ* hybridization, and immunohistochemistry), where basophilic intranuclear inclusions are commonly observed, as well as cytomegaly and karyomegaly.
- Polymerase chain reaction (PCR) assays have been developed, as well as enzyme-linked immunosorbent assays (ELISAs) for antibody detection.

Immunity

- There is no vaccine for PCMV.
- Seroconversion does not occur in piglets with congenital or neonatal infection.

Prevention and Control

- Preventing PCMV is challenging since nearly all swine are infected and disease is usually mild and difficult to recognize.
- To minimize the risk of PCMV transmission through xenotransplantation, donor pigs should be delivered via Cesarean section and specified pathogen-free or designated pathogen-free breeding practices should be used.

Gaps in Preparedness

- PCMV does not cause severe losses in swine.
- Further research is needed on the virus' zoonotic potential.
- No vaccines are available. It has been suggested that a vaccine could be used to eliminate PCMV from swine herds.

OVERVIEW

Porcine cytomegalovirus (PCMV) is an enveloped DNA virus belonging to the family *Herpesviridae* and the subfamily *Betaherpesvirinae*. Infection with PCMV has previously been known as 'inclusion body rhinitis' based on the histopathological characteristics of the disease. PCMV is endemic in almost all swine populations worldwide.

Swine are the natural host for PCMV. Direct contact and congenital transmission are known to occur. Disease is typically subclinical to mild, although high morbidity and mortality can be observed in neonates that develop systemic disease. Clinical signs in piglets may include shivering, sneezing, respiratory distress, poor weight gain, and rhinitis, as well as neurological signs. Like other herpesviruses, latent PCMV infections can develop and become reactivated when pigs are stressed.

Although no cases of PCMV have been documented in humans, concern exists about pathogen transmission through xenotransplantation. Research is mixed and it remains unclear whether PCMV is able to infect human cells.

Traditionally, PCMV is diagnosed via histological examination of tissue sections (staining, *in situ* hybridization, and immunohistochemistry), where basophilic intranuclear inclusions are commonly observed, as well as cytomegaly and karyomegaly. Polymerase chain reaction (PCR) assays have been developed, as well as enzyme-linked immunosorbent assays (ELISAs) for antibody detection. There is no vaccine for PCMV.

Preventing PCMV is challenging, as nearly all swine are infected and disease is usually mild and difficult to recognize. To minimize the risk of PCMV transmission through xenotransplantation, donor pigs should be delivered via Cesarean section and specified pathogen-free or designated pathogen-free breeding practices should be used.

PCMV does not cause severe losses in swine. Further research is needed on the virus' zoonotic potential. It has been suggested that a vaccine could be used to eliminate PCMV from swine herds.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Porcine cytomegalovirus (PCMV) is an enveloped DNA virus belonging to the family *Herpesviridae* and the subfamily *Betaherpesvirinae*.[1] The virus is also known as suid herpesvirus-2 (SuHV-2). Infection with PCMV has previously been known as 'inclusion body rhinitis' based on the histopathological characteristics of the disease.[1]

Currently, PCMV is not assigned to a particular genus. However, phylogenetic analysis suggests that PCMV could be classified as a member of the genus *Roseolovirus* in the subfamily *Betaherpesvirinae*.[2] Based on sequencing of the gB and major capsid protein genes, PCMV is closely related to human herpeseviruses 6 and 7, which are also members of the genus *Roseolovirus*.[3,4]

Like other herpesviruses, PCMV is composed of a core containing the DNA genome, an icosahedral capsid, and a lipid envelope containing embedded viral glycoproteins which act as major immunogens.[1] Glycoproteins (gB, gH, and gL) function in virus binding and fusion, respectively.[1] A recent genomic analysis found that PCMV is composed of nearly 130,000 bp containing 79 open reading frames (ORFs).[2]

Latent PCMV infection can develop in pigs that appear to have recovered from the disease, with monocytes/macrophages and CD8+ cells harboring the virus.[1,5] Viral recrudescence is related to stress and has been induced experimentally in pigs treated with corticosteroids.[6] Experimentally, it has been shown that more than 5500 genes are differentially expressed as a result of PCMV infection. [7]

1.2 Strain Variability

There are no distinctly separate PCMV serotypes or genotypes. However, some variations have been detected in isolates from different geographical areas. Researchers in Japan described antigenic variability among Japanese strains, and between Japanese strains and one isolated in the United Kingdom.[8] Another study identified variations in the gB gene in isolates from the United Kingdom, Germany, Spain, Japan, and Sweden.[4] In China, it has been suggested that two distinct sequence groups can be identified based on the gB gene.[9]

2. Cleaning and Disinfection

2.1 Survival

Little is known about the survival of PCMV in the environment. Subzero temperatures do not seem to impact the infectivity of the virus.[1]

2.2 Disinfection

Chloroform and ether inactivate PCMV.[1] Povidone-iodine (7.5%) is effective against human cytomegalovirus (human herpesvirus 5).[10] Most herpesviruses are also susceptible to 30% ethanol and isopropanol, 1% sodium hypochlorite, formaldehyde, 0.12% ortho-phenylphenol, and 0.04% glutaraldehyde.[10]

3. Epidemiology

3.1 Species Affected

Swine are the natural host for PCMV.

3.2 Zoonotic Potential
No cases of natural PCMV infection have been reported in humans. However, concern remains regarding potential transmission through xenotransplantation. Pig-to-primate transmission has been achieved and associated with coagulaopathy.[11-13] Experimentally, it has been shown that PCMV can infect human fibroblasts.[14] Other studies have failed to demonstrate cross-species transmission.[15,16] It remains unclear whether or not PCMV is able to infect human cells.[17]

3.3 Geographic Distribution
PCMV is found in swine populations throughout the world.

3.4 Morbidity and Mortality
PCMV is endemic in almost all swine populations. In Europe, North America, and Japan, more than 98% of swine are seropositive.[1] Recent data from China show that in Sichuan Province, nearly 85% of pigs show evidence of infection.[9] The reported seroprevalence in pigs from Hunan Province is 96%, with breeding sows most affected.[18]

Although most infections are subclinical to mild, fetal and neonatal death can occur in swine. Morbidity following congenital or neonatal infection is reported to be 100%.[1] Mortality is typically low, but co-infections can result in losses up to 50%.[1] Two studies have shown that use of PCMV-infected pig tissues for xenotransplantation appears to decrease survival time of the recipient (baboons or cynomolgus monkeys).[19,20]

4. Transmission
PCMV is shed in nasal secretions, with peak viral shedding at 5–8 weeks of age.[21] The virus can also be found in ocular secretions, as well as urine and cervical fluid.[1] PCMV spreads primarily via oronasal contact, but congenital transmission also occurs.[1]

5. Infection in Swine/Pathogenesis
After infection, PCMV primarily replicates in the nasal mucosa and/or lacrimal glands. This is followed by a cell-associated viremia two to three weeks post-infection and shedding of infectious virus in nasal secretions for a 10–30 day period. Secondary viral replication sites vary with the age of the individual. In nursery and growing pigs, PCMV has a tropism for nasal mucosal glands, lacrimal glands, kidney tubules, and, rarely, the epididymis and mucous glands of the esophagus. Fetal and neonatal pigs exhibit replication in the capillary endothelium and sinusoids of lymphatic tissues. These account for the systemic spread of PCMV and the presence of generalized lesions in very young piglets.[1]

5.1 Clinical Signs
Clinical signs are rare in pigs of all ages except young piglets, which are susceptible to developing fatal systemic disease. Neonates may die without exhibiting any clinical signs, although shivering, sneezing, respiratory distress, poor weight gain, and rhinitis have been observed.[1] Conjunctival discharge and black discoloration around the eyes has also been reported. Although PCMV has been suspected of contributing to periweaning failure-to-thrive syndrome (PFTS), recent evidence suggests that a role for the virus is unlikely.[22] Neurological signs can also be observed with PCMV.[1]

In pigs older than 3 weeks, disease is usually subclinical to mild. PCMV is associated with porcine respiratory disease complex (PRDC), and a correlation between PCMV and porcine circovirus-2 infection has been found among cases.[1,23]

Infection in pregnant sows can lead to mummified or stillborn piglets, and those that are born alive may be weak and underweight. In subsequent cycles, conception rate and litter sizes may be reduced.[24]

5.2 Postmortem Lesions

Gross lesions may only be seen in neonates. Catarrhal rhinitis, hydrothorax, hydropericardium, pulmonary and subcutaneous edema, and renal petechiation have been reported.[1] Fetal infection can result in stillbirth, mummification, embryonic death, and infertility.[1]

Characteristic histologic lesions include basophilic intranuclear inclusion bodies in cytomegalic cells of the nasal mucosa. Herpesvirions are visible by electron microscopy in epithelial cells of mucous glands in the nasal mucosa and salivary and lacrimal glands.[1] Inclusion bodies can also be seen in the CNS, particularly in the choroid plexus, cerebellum, and olfactory lobes.[1] In neonates with systemic disease, basophilic inclusions are seen in the capillary endothelium and sinusoidal cells of the lymphoid tissue.[1] Hemorrhage and edema also can occur due to vascular damage. Mononuclear cells and macrophages with inclusions can be seen in the blood vessels, alveoli, and spleen. Interstitial nephritis and hepatocellular necrosis have also been reported.[1]

6. Diagnosis

6.1 Clinical History

PCMV infection is usually mild to subclinical except in neonates, where infection may be suspected in cases of acute fatal systemic disease. In cases of respiratory disease or reproductive failure in older pigs, PCMV must be differentiated from a number of other diseases including classical swine fever, enterovirus, parvovirus, porcine reproductive and respiratory syndrome (PRRS), porcine circovirus-2 (PCV2), and pseudorabies.[1]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Virus isolation can be done in swine testicular cells[25], 19-PFT cells[26] (derived from pig fallopian tubes), and other primary and immortalized cell lines.[1] PCMV can be identified via histologic examination of tissue sections (staining, *in situ* hybridization, and immunohistochemistry), where basophilic intranuclear inclusions are commonly observed, as well as cytomegaly and karyomegaly.[1,27]

The first polymerase chain reaction (PCR) assay to detect PCMV was described in 1999.[28] Since then, a quantitative-competitive PCR (QC-PCR) has been described[29], as well as a multiplex PCR assay that detects PCMV, pseudorabies, and PCV2.[30] Another nucleic acid detection method, loop-mediated isothermal amplification (LAMP), has also been developed for PCMV.[31]

6.3 Tests to Detect Antibody

The enzyme-linked immunosorbent assay (ELISA) is useful for confirming PCMV infection in a herd of grower-finishers. An ELISA was first described in 1982[32], and highly sensitive ELISAs developed since then have shown that PCMV is widespread.[33] A recently developed indirect-blocking ELISA utilizes expressed major gB epitope as a coating antigen for the detection of PCMV antibodies, and has also been shown to be highly specific and sensitive.[34] Because *in utero* infection does not induce an antibody response, serological tests are not applicable to neonates that have been colostrum-deprived.[1]

6.4 Samples

6.4.1 Preferred Samples

Nasal swabs or scrapings and whole blood are the antemortem samples of choice. Appropriate postmortem samples include turbinate mucosa, lungs, pulmonary macrophages (obtained via lung lavage), and kidneys. Following reproductive failure, virus may be found in the brain, liver, or bone marrow of fetuses.[1]

6.4.2 Oral Fluids
The use of oral fluids for PCMV diagnosis has apparently not been investigated.

7. Immunity

7.1 Post-exposure
Antibodies are detectable by IFA 2–3 weeks after inoculation; levels peak at about 6 weeks but remain high for 10–11 weeks.[17] Seroconversion has not been observed in piglets with congenital or neonatal infection, although they do excrete virus and develop fatal systemic disease. Maternal antibody, which persists for about 2 months, provides some protection but does not prevent virus shedding on PCMV-endemic farms.[1]

7.2 Vaccines
Currently, there is no vaccine for PCMV. It has been suggested that the gB glycoprotein is a potential vaccine antigen candidate.[17]

7.3 Cross-protection
There are no separate PCMV serotypes or genotypes.

8. Prevention and Control
Disease is usually mild and difficult to recognize. It is challenging to prevent infection with PCMV. Introduction of new stock is a risk factor for PCMV, due to the possibility of reactivating latent infection or introducing disease to a PCMV-free herd.[1] Industry biosecurity practices, such as cleaning and disinfection between groups, should be in place. There is no treatment for PCMV, although antibiotics may be useful in treating secondary infections if present.

To prevent PCMV infection through xenotransplantation, it is recommended that donor pigs be delivered via Cesarean section and that specified pathogen-free or designated pathogen-free breeding practices are used.[17] Pigs can potentially be treated with anti-viral drugs to reduce viral loads (e.g., ganciclovir, cidofovir).[17] Anti-viral drugs may also be used to treat PCMV infection in xenograft recipients.[35] Vaccination has been suggested to eliminate PCMV from swine herds, although no vaccine currently exists.[17]

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code
PCMV is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no current recommendations on importation of swine or pork.

10. Gaps in Preparedness
PCMV does not cause severe losses in swine. Because of the virus' ubiquity and the planned future use of swine tissues in xenotransplantation to humans, the potential for zoonotic transmission should be further investigated. Information on latent infections is also sparse. The potential for vaccines to eliminate PCMV from swine herds should also be investigated.

ACKNOWLEDGEMENTS

Funding for this project was provided by the Swine Health Information Center, Perry, Iowa

Authors, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Kristin Killoran, PhD; 2nd year student
- Kerry Leedom Larson, DVM, MPH, PhD; Veterinary Specialist

Reviewers, Center for Food Security and Public Health, College of Veterinary Medicine, Iowa State University:

- Pamela Zaabel, DVM; Veterinary Specialist
- James A. Roth, DVM, PhD; Director

To cite:
Killoran K, Leedom Larson KR. Porcine cytomegalovirus. Swine Health Information Center and Center for Food Security and Public Health, 2016. http://www.cfsph.iastate.edu/pdf/shic-factsheet-porcine-cytomegalovirus.

REFERENCES

1. *Diseases of Swine.* 10th ed. Ames, IA: Wiley-Blackwell; 2012.
2. Gu W, Zeng N, Zhou L, Ge X, Guo X, Yang H. Genomic organization and molecular characterization of porcine cytomegalovirus. *Virology.* 2014;460-461:165-172.
3. Rupasinghe V, Iwatsuki-Horimoto K, Sugii S, Horimoto T. Identification of the porcine cytomegalovirus major capsid protein gene. *J Vet Med Sci.* 2001;63(6):609-618.
4. Widen F, Goltz M, Wittenbrink N, Ehlers B, Banks M, Belak S. Identification and sequence analysis of the glycoprotein B gene of porcine cytomegalovirus. *Virus Genes.* 2001;23(3):339-346.
5. Guedes MI, Risdahl JM, Wiseman B, Molitor TW. Reactivation of porcine cytomegalovirus through allogeneic stimulation. *J Clin Microbiol.* 2004;42(4):1756-1758.
6. Edington N, Watt RG, Plowright W. Cytomegalovirus excretion in gnotobiotic pigs. *J Hyg (Lond).* 1976;77(2):283-290.
7. Liu X, Xu Z, Zhu L, Liao S, Guo W. Transcriptome analysis of porcine thymus following porcine cytomegalovirus infection. *PLoS One.* 2014;9(11):e113921.
8. Tajima T, Kawamura H. Serological relationship among porcine cytomegalovirus Japanese isolates and a UK isolate. *J Vet Med Sci.* 1998;60(1):107-109.
9. Liu X, Liao S, Zhu L, Xu Z, Zhou Y. Molecular epidemiology of porcine cytomegalovirus (PCMV) in Sichuan Province, China: 2010-2012. *PLoS One.* 2013;8(6):e64648.
10. Public Health Agency of Canada. Pathogen Safety Data Sheet: Cytomegalovirus. 2011; http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/cytomegalovirus-eng.php. Accessed December 18, 2015.
11. Mueller NJ, Barth RN, Yamamoto S, Kitamura H, Patience C, Yamada K, Cooper DK, Sachs DH, Kaur A, Fishman JA. Activation of cytomegalovirus in pig-to-primate organ xenotransplantation. *J Virol.* 2002;76(10):4734-4740.
12. Gollackner B, Mueller NJ, Houser S, Qawi I, Soizic D, Knosalla C, Buhler L, Dor FJ, Awwad M, Sachs DH, Cooper DK, Robson SC, Fishman JA. Porcine cytomegalovirus and coagulopathy in pig-to-primate xenotransplantation. *Transplantation.* 2003;75(11):1841-1847.
13. Mueller NJ, Kuwaki K, Dor FJ, Knosalla C, Gollackner B, Wilkinson RA, Sachs DH, Cooper DK, Fishman JA. Reduction of consumptive coagulopathy using porcine cytomegalovirus-free cardiac porcine grafts in pig-to-primate xenotransplantation. *Transplantation.* 2004;78(10):1449-1453.
14. Whitteker JL, Dudani AK, Tackaberry ES. Human fibroblasts are permissive for porcine cytomegalovirus in vitro. *Transplantation.* 2008;86(1):155-162.
15. Plotzki E, Wolf-van Buerck L, Knauf Y, Becker T, Maetz-Rensing K, Schuster M, Baehr A, Klymiuk N, Wolf E, Seissler J, Denner J. Virus safety of islet cell transplantation from transgenic pigs to marmosets. *Virus Res.* 2015;204:95-102.
16. Garkavenko O, Dieckhoff B, Wynyard S, Denner J, Elliott RB, Tan PL, Croxson MC. Absence of transmission of potentially xenotic viruses in a prospective pig to primate islet xenotransplantation study. *J Med Virol.* 2008;80(11):2046-2052.
17. Denner J. Xenotransplantation and porcine cytomegalovirus. *Xenotransplantation.* 2015;22(5):329-335.
18. Liu GH, Li RC, Li J, Huang ZB, Xiao CT, Luo W, Ge M, Jiang DL, Yu XL. Seroprevalence of porcine cytomegalovirus and sapovirus infection in pigs in Hunan province, China. *Arch Virol.* 2012;157(3):521-524.
19. Yamada K, Tasaki M, Sekijima M, Wilkinson RA, Villani V, Moran SG, Cormack TA, Hanekamp IM, Hawley RJ, Arn JS, Fishman JA, Shimizu A, Sachs DH. Porcine cytomegalovirus infection is associated with early rejection of kidney grafts in a pig to baboon xenotransplantation model. *Transplantation.* 2014;98(4):411-418.

20. Sekijima M, Waki S, Sahara H, Tasaki M, Wilkinson RA, Villani V, Shimatsu Y, Nakano K, Matsunari H, Nagashima H, Fishman JA, Shimizu A, Yamada K. Results of life-supporting galactosyltransferase knockout kidneys in cynomolgus monkeys using two different sources of galactosyltransferase knockout swine. *Transplantation.* 2014;98(4):419-426.
21. Plowright W, Edington N, Watt RG. The behaviour of porcine cytomegalovirus in commercial pig herds. *J Hyg (Lond).* 1976;76(1):125-135.
22. Huang Y, Gauvreau H, Harding J. Diagnostic investigation of porcine periweaning failure-to-thrive syndrome: lack of compelling evidence linking to common porcine pathogens. *J Vet Diagn Invest.* 2012;24(1):96-106.
23. Hansen MS, Pors SE, Jensen HE, Bille-Hansen V, Bisgaard M, Flachs EM, Nielsen OL. An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark. *J Comp Pathol.* 2010;143(2-3):120-131.
24. Smith KC. Herpesviral abortion in domestic animals. *Vet J.* 1997;153(3):253-268.
25. Shirai J, Narita M, Iijima Y, Kawamura H. A cytomegalovirus isolated from swine testicle cell culture. *Nihon Juigaku Zasshi.* 1985;47(5):697-703.
26. Kawamura H, Tajima T, Hironao T, Kajikawa T, Kotani T. Replication of porcine cytomegalovirus in the 19-PFT cell line. *J Vet Med Sci.* 1992;54(6):1209-1211.
27. Sekiguchi M, Shibahara T, Miyazaki A, Tajima T, Shimizu S, Kabali E, Takano Y, Sasaki Y, Kubo M. In situ hybridization and immunohistochemistry for the detection of porcine cytomegalovirus. *J Virol Methods.* 2012;179(1):272-275.
28. Hamel AL, Lin L, Sachvie C, Grudeski E, Nayar GP. PCR assay for detecting porcine cytomegalovirus. *J Clin Microbiol.* 1999;37(11):3767-3768.
29. Fryer JF, Griffiths PD, Fishman JA, Emery VC, Clark DA. Quantitation of porcine cytomegalovirus in pig tissues by PCR. *J Clin Microbiol.* 2001;39(3):1155-1156.
30. Lee CS, Moon HJ, Yang JS, Park SJ, Song DS, Kang BK, Park BK. Multiplex PCR for the simultaneous detection of pseudorabies virus, porcine cytomegalovirus, and porcine circovirus in pigs. *J Virol Methods.* 2007;139(1):39-43.
31. Yang JL, Zhang SH, Liu ZH, Yang R, Huang Y, Wen M. Development and evaluation of a loop-mediated isothermal amplification assay for the rapid detection of porcine cytomegalovirus under field conditions. *Virol J.* 2012;9:321.
32. Assaf R, Bouillant AM, Di Franco E. Enzyme linked immunosorbent assay (ELISA) for the detection of antibodies to porcine cytomegalovirus. *Can J Comp Med.* 1982;46(2):183-185.
33. Tajima T, Hironao T, Kajikawa T, Kawamura H. Application of enzyme-linked immunosorbent assay for the seroepizootiological survey of antibodies against porcine cytomegalovirus. *J Vet Med Sci.* 1993;55(3):421-424.
34. Liu X, Zhu L, Shi X, Mei M, Xu W, Zhou Y, Guo W, Wang X. Indirect-blocking ELISA for detecting antibodies against glycoprotein B (gB) of porcine cytomegalovirus (PCMV). *J Virol Methods.* 2012;186(1-2):30-35.
35. Fryer JF, Griffiths PD, Emery VC, Clark DA. Susceptibility of porcine cytomegalovirus to antiviral drugs. *J Antimicrob Chemother.* 2004;53(6):975-980.