In situ hybridization and immunohistochemistry for porcine cytomegalovirus detection in paraffin-embedded tissue sections

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Introduction
Porcine cytomegalovirus (PCMV) is a member of the β-herpesvirus that has the worldwide distribution (1). PCMV causes inclusion body rhinitis in swine up to 10 weeks of age, most severely in swine less than 2 weeks old, and thereafter the infection is subclinical. PCMV is also one of the pathogens that should be eliminated from pigs intended for use as organ donors in xenotransplantation. PCMV detection methods include serology, viral culture, polymerase chain reaction (PCR), and histopathological examination [hematoxylin and eosin (HE), immunohistochemistry (IHC) and in situ hybridization (ISH)]. ISH and IHC would enable more sensitive detection of PCMV in tissue sections compared with routine HE staining. Until recently, IHC was, however, rarely performed, and ISH was not. We compared the PCMV results of an ISH procedure and an IHC technique to that previously evaluated cases for PCMV and to each other.

Materials and Methods
The study comprised 35 piglets from 16 farms submitted for necropsy between 2005 and 2010. The piglets were suspected with PCMV infection by PCR or histological examination. For histopathological examination, the formalin-fixed paraffin-embedded renal tissue sections (3 µm) were stained with HE. For IHC, serial sections were prepared with the universal immunoenzyme polymer method using a Histofine simple stain MAX-PO Kit (Nichirei Corp., Tokyo, Japan). The sections were pretreated with 0.1% actinase and endogenous peroxidase activity was blocked by 3% H2O2 in methanol. The primary antibody was a rabbit polyclonal antibody specific for PCMV JI NP-40 applied at a dilution of 1 in 1024. The sections were lightly counterstained with hematoxylin and assessed by light microscopy. ISH was performed using a MicroProbe Staining system (Falma, Tokyo Japan). Briefly, after deparaffin, followed by enzymatic digestion with pepsin, mRNA in sections were hybridized for 23 hours at 37°C with the digoxigenin (DIG)-labelled PCMV-specific cRNA probe targeting glycoprotein B gene. The sequences of the probe were as follows; 5’-

TTTGATATGTATCGGTCTTTCACGAG
ATATAAATGCTTGTTTGCAGATAGTGAG
GGCTGGTGGAGTTTGAATCTAAAAT-3’. After post hybridization, the DIG-labelled hybrids were sequentially detected with an alkaline phosphatase-conjugated anti-DIG antibody (Roche, Tokyo, Japan) with Stable Fast Red/Naphtol Phosphate.

Results
By HE, PCMV inclusion bodies were found in the nucleus of renal epithelial cells, and endothelial cells of capillary in medulla of kidney. The cells were characterized by cytomegaly, basophilic intranuclear inclusion bodies. 31/35 (88.6%) cases were PCMV-positive, whereas 4 were negative. By IHC, PCMV antigens were mainly and clearly detected in the cytoplasm of the cells. 22/35 (62.9%) cases were PCMV-positive, 13 were negative. By ISH, PCMV signals were mainly and clearly detected in the nucleus of the cells. 29/35 (82.9%) cases were PCMV-positive, 6 were negative.

Discussion
ISH and IHC were more clear detection methods of PCMV in tissue sections compared with routine HE staining. These methods have high specificities and strong stain intensity with minimal background. We recommend either ISH or IHC for PCMV detection in formalin-fixed paraffin-embedded tissues. When compared to ISH, the positive rate of IHC was low. Possible antigenic variability has been reported (1). The present results also indicate the possibility of antigenic variation among PCMV strains.

References

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