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Multiple models of porcine teschovirus pathogenesis in endemically infected pigs

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ABSTRACT

Porcine teschoviruses (PTVs) belong to the genus *Teschovirus* within the family *Picornaviridae*. PTVs are universal contaminants in pig herds in endemic and multi-infection status. To further the understanding of PTV pathogenesis in endemically infected pigs, a set of samples was studied by real time reverse transcription PCR (qRT-PCR) to quantitate viral loads in tissues and by in situ hybridization (ISH) to locate PTV signals in target cells, both targeting the 5′-NTR. cRNA of PTV-1 and PTV-7, in vitro transcribed from cloned fragments of 5′-NTR of 2 viruses, was used to construct standard curves and to run parallel in qRT-PCR, which had detection limits of 10^1 copies/per reaction, with a linearity in between 10^1 and 10^7 copies/per reaction and correlation coefficients of 0.997–0.9988. The qRT-PCR specifically amplified RNA from PTV-1 to -11, while excluding those of *Sapelovirus*, PEV-9 and PEV-10. Inguinal lymph node (LN) had the highest viral load of all (assuming 100%), followed by ileac LN (89–91%), tonsil (66–68%), ileum (59–60%), spleen (38–40%), and kidney (30–31%), with the least in brain (22.9%) of the inguinal LN. The 22.9% load in brain was higher than that anticipated from a simple fecal-oral-viremia operative model. The results suggested in addition that intranasal infection and retrograding axonal infection from the tonsils were equally operative and significant. ISH revealed PTV signals in a wider variety of tissue cell types than before. PTV signals were noted most impressively in neurons of the cerebral cortex and hippocampus and in the dark zone of the germinal center and adjacent paracortex of regional LN. Multiple operative models indicated that PTVs seemed to have no difficulty invading the brain. The key to whether encephalitis would ensue resided in the animal's immune status and topographic differences of neurons' susceptibilities to PTVs. When common co-infected agents are present, as is typical in the field, PTVs may synergize in causing diseases.

1. Introduction

Porcine teschoviruses (PTVs) belong to the genus *Teschovirus* within the family *Picornaviridae*. The virions are spherical, nonenveloped, and 25–30 nm in diameter, and they contain a linear plus sense single-stranded ribonucleic acid (ssRNA) genome surrounded by an icosahedral capsid (Knowles, 2006). The original genus *Enterovirus* was reclassified into 3 groups: *Teschovirus* (CPE group I, PEV types 1–7 and 11–13), *Sapelovirus* (Porcine Enterovirus A, CPE group II, PEV-8), and Porcine Enterovirus B (CPE group III, PEV-9 and PEV-10) (ICTV, 2012; Kaku et al., 2001; Zell et al., 2001). Currently PTVs have a total of 13 serotypes associated with a variety of clinical conditions including polioencephalomyelitis, female reproductive disorders, enteric disease, and pneumonia

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(Knowles, 2006; Cano-Gómez et al., 2011b; Boros et al., 2012; Deng et al., 2012). Recently via phylogenetic tree analysis a PTV-12 was identified in domestic pigs (Cano-Gómez et al., 2011b) and a PTV-13 in wild boars (Boros et al., 2012).

In the past, a similar clinical to those of Talfan disease was observed in two waves of PTV-1 epidemics in 2000 and 2004. Moreover PTVs were found to be co-infected with common swine pathogens such as PCV2, PRRSV and CSFV, reflecting the multi-infection status in the field (Table 1 in Chiu et al., 2012). Since then PTVs have been isolated from pigs of different growing stages with varying signs of respiratory, enteric, neurologic, and reproductive disorders (Huang et al., 2009). Nowadays PTV has become endemic in swine herds (Knowles, 2006; Qiu et al., 2013) and few herds or pigs are really PTV free. Co-infection of at least 2 PTV serotypes in the same animals or herds (Chiu et al., 2012) and co-circulation of several serotypes in a geographic area (Cano-Gómez et al., 2011b) are common. A previous study indicated that PTV pathogenesis can be staged by studying tissues/organs from 4 major groups (brain, viscera, lymphoid tissues, and intestines), in which the intestines had the highest PTV detection rate, followed by lymphoid tissues, viscera and the brain (Chiu et al., 2012). These results seemed consistent with the fecal-oral model of pathogenesis, although some variation was present; for example, the cranial and medial portions of the brain (Br–Cr) had higher detection rates than that of the caudal portion (Table 4 in Chiu et al., 2012). However, only infection in the caudal portion of the brain (Br–Ca), despite that portion being exposed to relatively lower amounts of PTV (reflected by a lower detection rate by nested PCR), was significantly correlated with the nonsuppurative encephalitis presented therein. To further the understanding of PTV pathogenesis in the field, the aim of this study was to analyze a set of samples from endemically infected pigs by real-time reverse transcription PCR (qRT-PCR) and by in situ hybridization (ISH) both targeting the 5′-NTR. The results revealed in this study provide more direct evidence to substantiate the multiple operative models of PTV pathogenesis in endemically infected pigs.

2. Materials and methods

2.1. Experimental design

This study utilized qRT-PCR (Jimenez-Clavero et al., 2003) to absolutely quantitate the PTV load in tissues. For a fair comparison with positive sense PTV RNA, positive sense cRNA (Krumbholz et al., 2003) from two viruses, PTV-1 (AHRI) and PTV-7 (WR2, NVSL) (Chiu et al., 2012), were used to construct standard curves and run in parallel with tissue total RNA during qRT-PCR. PTV-1 was selected because it caused two waves of epidemics in years 2000 and 2004. PTV-7 was selected because it was one of the prevalent serotype in a previous study (Chiu et al., 2012).

The cRNA standard was obtained by extracting viral RNA of standard viruses, which was reverse transcribed, electrophoresed, extracted from gel, and cloned. Plasmid DNA of the desired clones was then used for transcription in vitro to produce positive sense cRNA, whose quantity

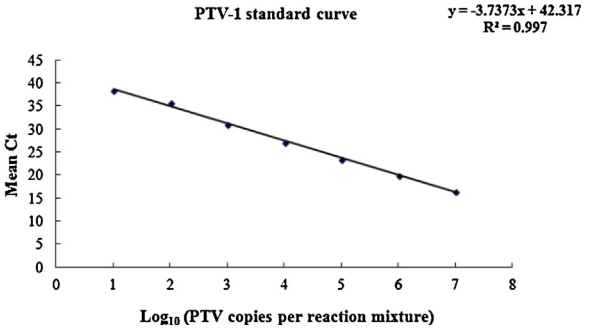

Fig. 1. Linear regression line of PTV-1 (AHRI) as standard in qRT-PCR. Equation showed a correlation coefficient of 0.997, slope of −3.7373, and efficiency of 1.830.

was measured and converted to copy number per μL by the formula of Fronhoffs et al. (2002) (detailed in supplementary file). To test for the sensitivity (detection limit) and to construct the standard curves, cRNA was serially diluted from 10^7 to 10^0 copies per reaction (Figs. 1 and 2; Table 2; supplementary). To test for the specificity of the qRT-PCR, a number of reference strains (Fig. 3, Table 1) were used. To test for viral loads in tissues, 10^7 copies per reaction of cRNA and 1 μg of tissue total RNA per reaction in triplicate were run in parallel through the procedures (Tables 3–5). To locate 5′-NTR signals in tissues or cell types, formalin-fixed paraffin-embedded (FFPE) tissues from the same group of animals were analyzed by ISH (Fig. 4, Table 6).

2.2. Sampling of tissues

Culled post-weanling piglets ($n = 29$), aged from 4 to 8 weeks, were collected from 2 out of 3 farrow-to-finish herds. The herd background and the sampling plan were as in Chiu et al. (2012). The set of organs included cranial and medial portions of the cerebrum (Br–Cr), kidney, spleen, tonsil, ileac lymph node (LN), inguinal LN, and ileum.

2.3. Preparation of tissue total RNA

Excised tissues, approximately 100 mg of cerebrum (included cranial and medial parts, Br–Cr) and 35–40 mg of kidney, spleen, tonsil, ileal LN, ileum, and inguinal LN,

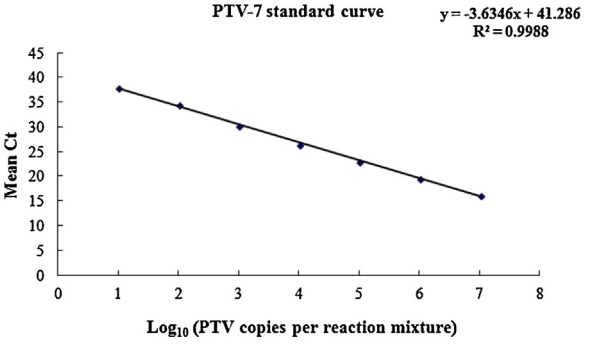

Fig. 2. Linear regression line of PTV-7 (WR2, NVSL) as standard in qRT-PCR. Equation showed a correlation coefficient of 0.9988, slope of −3.6346, and efficiency of 1.955.