Veterinary Microbiology 182 (2016) 150–155

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The urinary shedding of porcine teschovirus in endemic field situations

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ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 14 October 2015
Accepted 12 November 2015

Keywords:
Porcine teschovirus
Viral load
qRT-PCR
Pathogenesis
Plasma
Urine
Nasal swab
Molecular serotyping

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 statuses. Previous research has demonstrated PTV antigens and nucleic acid in renal glomeruli and tubular epithelia, suggesting the possibility that PTVs might be shed and transmitted via urine. The study aimed to demonstrate, in the context of pathogenesis, the presence of PTVs in the urine of naturally infected pigs. Viral loads of fluid and tissue samples quantified by an established qRT-PCR showed detection rates of 100% by head and in urine, feces, plasma and nasal swabs, and 38% in kidney. As predicted, PTVs were present in urine at $10^{4.02 \pm 1.45}$ copies/100 μl volume, equivalent to 17% of that in plasma. No significant differences were observed between healthy and culled pigs or among the 7 sampled herds. The presence of PTVs in urine was further substantiated by molecular serotyping. In particular, PTV-10 was identified in the urine of 3 piglets from 3 separate herds, consistent with the most prevalent serotype found in this study, and in plasma. The urine mixes with feces to form slurry making it easier for PTV to spread and contaminate the environment.

1. Introduction

Porcine teschovirus (PTV) is a non-enveloped, positive-sense, single-strand RNA virus of 25–30 nm in diameter belonging to the genus *Teschovirus* within the family *Picornaviridae*. To date, at least 13 serotypes of PTV (PTV-1 to -13) have been identified (Alexandersen et al., 2012; Boros et al., 2012; Cano-Gómez et al., 2011b; Kaku et al., 2001; Zell et al., 2001). The clinical signs, which vary with the different serotypes and virulence of PTVs, include neurological disorders (mainly polioencephalomyelitis), enteric diseases, female reproductive disorders, and pneumonia. The virulent PTV-1 strains are associated with severe nonsuppurative encephalomyelitis, called Teschen disease, which caused epidemics in 1930–1950 in Europe and Africa (Horstmann, 1952). The less virulent PTV-1 strains are associated with mild encephalomyelitis, called Talfan disease, which today is maintained predominantly in herds (Alexandersen et al., 2012; Chiu et al., 2012; Chiu K.C. et al., 2014; Chiu S.C. et al., 2014).

The transmission of PTVs primarily follows the fecal–oral route (Kanamitsu et al., 1967; Racaniello, 2006). After viral particles are ingested, PTV initially replicates in the tonsils and intestinal tract, and then it spreads to regional lymph nodes, blood, and visceral organs. The virus reaches the central nervous system mainly by viremia (Alexandersen et al., 2012) and/or retrograde axonal transport (Chiu K.C. et al., 2014; Chiu S.C. et al., 2014). In endemic situations, pigs are usually asymptomatic (Buitrago et al., 2010; Sozzi et al., 2010). Visceral organs such as the spleen and kidneys, which function to filter blood antigens and metabolic wastes, may become infected by PTV during viremia (Chiu K.C. et al., 2014; Chiu S.C. et al., 2014), as indicated by the finding of PTV antigen and nucleic acid signals in the kidneys (Chiu K.C. et al., 2014; Chiu S.C. et al., 2014). Epithelia of the renal tubules and urinary bladder may exfoliate and regenerate, and the flow of glomerular infiltrate, which later becomes urine, is unidirectional. It is thus reasonable to speculate that PTV may be shed in the urine. However, little evidence has been available to verify this speculation. The purpose of this study was to demonstrate the urinary shedding of PTVs.

2. Materials and methods

2.1. Experimental design

Twenty-eight post-weaned pigs, 7 clinically healthy (per owner) and 21 culled, were obtained from 7 herds. All pigs were

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http://dx.doi.org/10.1016/j.vetmic.2015.11.008
0378-1135/

aged from 5 to 8 weeks, and their average body weight was 6.1 ± 1.6 kg. The culled piglets showed weakness, respiratory symptoms, emaciation, or poor feed conversion. Gross lesions, mostly of polyserositis, were apparently unrelated to PTV infection.

The samples were obtained from piglets immediately after electrical euthanasia. Nasal swab was included for comparison because a previous study indicated a higher detection rate of PTV in anterior portion, including olfactory bulb of the brain (Chiu et al., 2012). To avoid contamination from the slurry, nasal swabs were taken after the nostrils were cleaned. Swabs were inserted 2 cm deep into the nasal cavity, and each swab was immersed in 1 ml DEPC-treated PBS. Coagulation was prevented with EDTA (1.5 mg/ml blood). The urine was collected by paracentesis of the urinary bladder immediately after the opening of the abdominal cavity to avoid cross contamination from intestinal contents or slurry. Feces were taken directly from the rectum to avoid cross contamination to the urine. The rest of samples were taken in a sequence based on the predicted viral loads of low to high (Chiu et al., 2012; Chiu K.C. et al., 2014; Chiu S.C. et al., 2014), in order the urinary bladder, kidney, spleen, tonsil, ileum, and inguinal lymph node (LN), so as to reduce cross contamination.

2.2. Preparation of tissue total RNA

For solid tissues: Frozen tissues were micro-dissected into weights of approximately 30 mg for bladder and 25 mg for kidney, spleen, tonsil, ileum, and inguinal LN tissue. Total RNA was extracted using an RNeasy® mini kit (Qiagen, Germany) and quantitated as before (Chiu S.C. et al., 2014).

For fluid samples (diluted feces, plasma, urine, nasal swabs): Approximately 100 mg of the feces was mixed with 900 μl of DEPC-treated PBS and then centrifuged at 7000 × *g* for 10 min at 4 °C. The supernatant was filtered through a 0.22 μm filter. Plasma was obtained from the anticoagulated whole blood after centrifugation at 5000 × *g* at 4 °C for 15 min.

Two hundred and fifty microliters of the fecal supernatant, urine, plasma, and nasal swab samples was mixed with 750 μl of TRIzol® LS reagent (Invitrogen, USA) following the manufacturer's protocol. Then 20 μg of glycogen was added during RNA precipitation and the solution diluted in 50 μl of RNase-free water.

Relative RNA yields in solid tissues and fluid samples for correction of viral loads: The variety of textures of the solid tissues and fluid samples resulted in a wide range of RNA yields. The viral loads obtained for each sample were corrected according to their relative RNA yields as published in Chiu S.C. et al., (2014) and were expressed as total RNA (μg)/tissue weight (mg) or volume of fluid (μl) (Table 1). This assumed that the specific gravities of both solid tissues and fluid samples were roughly equivalent to 1 gm/cm^3 or ml, and that the weight differences among these samples were negligible relative to the log scale of the qRT-PCR.

2.3. Absolute quantitative real-time RT-PCR (qRT-PCR) for viral loads

An established qRT-PCR targeting the highly conserved 5′-NTR region (Chiu S.C. et al., 2014) modified from Cano-Gómez et al., (2011a) was used to quantitate the PTV loads in tissues and fluid samples. The assay was specific for PTV-1 to -11, while excluding sapelovirus, porcine enterovirus-9 and -10 tested. The assay had a sensitivity of 10^1 copies/reaction to PTV-7 (WR2) and variable sensitivities to other PTVs tested. The experiments were performed on a MyiQTM2 real-time PCR detection system in 8-tube strips (Bio-Rad) and the accompanying software.

The possible differences between healthy and culled pigs, among swine herds, and among types of samples, were analyzed using IBM SPSS Version 20 (SPSS Statistics V20, IBM Corporation, Somers, New York).

2.4. Pan-PTV nested RT-PCR to screen for PTV infection

The pan-PTV nested RT-PCR, targeted on 5′NTR, was executed as in Chiu et al., (2012) following Zell et al., (2000).

2.5. Nested RT-PCR on VP1 region for molecular serotyping

For VP1 amplifications, two degenerate primer pairs, PTV-SF/SR and PTV-nSF/nSR (Table 2) were designed by DNA star software (Lasergene), based on 28 available PTV nucleotide sequences representing all the PTV serotypes (PTV1-13) in GenBank (accession numbers: NC_003985, af231768-69, af296087-89, af296090-94, af296096, af296100, af296102, af296104, af296107-09, af296111-13, af296115, af296117-19, JN859128,

Table 1
Viral copy numbers per 100 mg tissues and 100 μl fluids in different samples (n = 28).

Samples	Feces	Ileum	Tonsil	Ig LN	Plasma	Spleen	Kidney	Bladder	Urine	Nasal[d]
Number of positive detection (Ct > 1)	28/28	27/28	23/28	21/28	28/28	12/28	11/28	18/28	28/28	28/28
Uncorrected Viral copy number per 1 μg total RNA (n = 28) (Geometric mean ± SD)	$10^{5.04 \pm 0.92}$	$10^{4.12 \pm 1.27}$	$10^{2.64 \pm 1.81}$	$10^{2.53 \pm 1.65}$	$10^{5.01 \pm 1.32}$	$10^{1.22 \pm 1.62}$	$10^{1.20 \pm 1.58}$	$10^{1.69 \pm 1.50}$	$10^{4.42 \pm 1.45}$	$10^{3.98 \pm 0.68}$
Average RNA yield (μg total RNA per 1 mg tissue or 1 μl fluid)	0.077	1.887	1.890	1.894	0.006	2.038	1.298	0.556	0.004	0.005
Relative RNA yield among samples	0.038	0.926	0.927	0.929	0.003	1.000	0.637	0.273	0.002	0.002
Corrected viral copy number per 1 μg total RNA[a,b] (Geometric mean ± SD)	$10^{3.62 \pm 0.92}$	$10^{4.09 \pm 1.27}$	$10^{2.61 \pm 1.81}$	$10^{2.50 \pm 1.65}$	$10^{2.49 \pm 1.32}$	$10^{1.22 \pm 1.62}$	$10^{1.00 \pm 1.58}$	$10^{1.13 \pm 1.50}$	$10^{1.71 \pm 1.45}$	$10^{1.37 \pm 0.68}$
Copy numbers per 100 mg or 100 μl specimens[c]	$10^{5.92 \pm 0.92}$	$10^{6.40 \pm 1.27}$	$10^{4.92 \pm 1.81}$	$10^{4.81 \pm 1.65}$	$10^{4.79 \pm 1.32}$	$10^{3.52 \pm 1.62}$	$10^{3.31 \pm 1.58}$	$10^{3.44 \pm 1.50}$	$10^{4.02 \pm 1.45}$	$10^{3.68 \pm 0.68}$
Arithmetic mean	831764	2511886	83176	64565	61660	3311	2042	2754	10471	4786
Relative viral load among samples (plasma as 100%)	1349%	4074%	135%	105%	100%	5.4%	3.3%	4.5%	17.0%	7.8%

Note: SD = standard deviation.

[a] The total number of specimens to average was 28, including 21 culled piglets and 7 outwardly looking healthy piglets from the same herds. Later statistical analysis showed no differences between culled and healthy piglets, so their data are presented together.

[b] The method of correction for average RNA yield (and therefore relative RNA yield among samples) are as published in Table 3 of Chiu S.C. et al. (2014).

[c] Copy numbers per 100 mg tissue (copy/mg) or 100 μl fluid specimens (copy/μl) = Uncorrected copy number per 1 μg total RNA (copy/μg) x average RNA yield (μg/mg or μg/μl) x 100 (mg tissue or μl fluid).

[d] Listed are the viral loads contained in a whole single swab.