Veterinary Microbiology 172 (2014) 367–370

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Detection and distribution of torque teno sus virus 1 in porcine reproductive and respiratory syndrome virus positive/negative pigs

Zhicheng Zhang [a,*], Yang Wang [b], Wei Dai [c], Dingzhen Dai [a]

[a] Department of Animal Science and Technology, Jinling Institution of Technology, Nanjing 210038, China
[b] College of Animal Science and Technology, Henan University of Science and Technology, Luoyang 471003, China
[c] Key Laboratory of Zoonoses of Anhui Province, Anhui Agricultural University, Hefei 230036, China

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form 3 May 2014
Accepted 5 May 2014

Keywords:
Torque teno sus virus 1
Porcine reproductive and respiratory syndrome virus
Virus detection
Virus distribution

ABSTRACT

To investigate the detection rate and distribution of torque teno sus virus 1 (TTSuV1) in porcine reproductive and respiratory syndrome virus (PRRSV) positive/negative pigs, 2384 pathological tissues samples collected from 6 provinces of Eastern China from 2010 to 2013 were amplified using previously published PRRSV and TTSuV1 primers. The presence and viral load of TTSuV1 were investigated in a wide range of samples from 5 PRRSV positive/negative 4-week-old pigs by real-time TaqMan PCR. TTSuV1 was detected in 65.3% of 1115 PRRSV-positive samples, and 47.2% of 1269 negative samples. Viral DNA was most commonly detected in the immune organs, including spleen, lung, pancreas, and mesenteric and inguinal lymph nodes, followed by serum, liver, kidney, trachea, anal swabs, nasal swabs and sex glands of PRRSV-positive or negative pigs. TTSuV1 DNA loads in PRRSV-positive pigs increased from 2 to 5 times in almost all the corresponding parts compared with PRRSV-negative pigs. Statistical analysis showed that PRRSV may have a synergistic effect with TTSuV1, and promote the replication and proliferation of TTSuV1.

1. Introduction

Torque teno virus (TTV) is a small, non-enveloped, single-stranded circular DNA virus (Okamoto et al., 2002), which was first discovered in serum of Japanese patients with acute post-transfusion hepatitis of unknown etiology in 1997 (Nishizawa et al., 1997; Okamoto et al., 1999a). Since then, TTV has been detected in many other vertebrate animals, non-human primates and domestic animals, such as pigs, chickens, cows, sheep, cats and dogs (Leary et al., 1999; Okamoto et al., 1999b, 2002). In swine and wild boar, two genetically distinct TTVs, torque teno sus virus 1 (TTSuV1) and TTSuV2, have been identified (Martelli et al., 2006; Niel et al., 2005). At present, there is no effective cell culture and serological detection methods for TTSuVs (Gallei et al., 2009, 2010).

Many studies have confirmed that TTSuV1 infection is linked to porcine circovirus 2 (PCV2) infection and promotes porcine dermatitis and nephropathy syndrome (Ellis et al., 2008; Kekarainen et al., 2006; Krakowka et al., 2008; Taira et al., 2009). In a gnotobiotic piglet infection model, TTSuV1 promotes both PCV2 and porcine reproductive and respiratory syndrome virus (PRRSV) infections (Krakowka and Ellis, 2008). PCV2 infection without manipulation does not cause clinical signs or post-weaning multisystemic wasting syndrome (PMWS), whereas, when PCV2 infection is combined with TTSuV1 infection, PMWS is produced. In addition, TTSuV1 co-infection with PRRSV is correlated with the development of porcine dermatitis and a nephropathy-syndrome-like condition in gnotobiotic

* Corresponding author. Tel.: +86 25 85393317.
E-mail address: andzzc@126.com (Z. Zhang).

http://dx.doi.org/10.1016/j.vetmic.2014.05.008
0378-1135/

pigs. Zhang et al. (2012) have found that TTSuV1 natural infection has an adverse effect on the development of host immune responses, suppresses immunization of PRRS MLV vaccine, and prompts PRRS exacerbation in pigs.

Currently, research on TTSuVs is mainly concentrated on detection methods and genomic characteristic analysis. No in-depth studies have so far been made on quantitative distribution in various organs and tissues of pigs during natural infection with TTSuVs. Also, there are no reports about the effect of co-infection with PRRSV on TTSuV1 distribution in various organs and tissues. The present study established the SYBR Green I real-time PCR method, to measure the relative content of TTSuV1 DNA in organs and tissues during natural infection in pigs [heart, liver, spleen, lung, kidney, pancreas, trachea, fat, intestine, mesenteric lymph nodes (MLNs), inguinal lymph nodes (ILNs), anal swabs (ASs), nasal swabs (NSs), ovaries and testes). The objective of this study was to investigate the detection rate and distribution of TTSuV1 in PRRSV positive/negative pigs.

2. Material and methods

2.1. Samples and animals

A total of 2384 infected pigs displaying clinical symptoms (such as respiratory signs and fever) were collected from 6 provinces (Shandong, Anhui, Zhejiang, Shanghai, Fujian, and Jiangsu) in Eastern China from 2009 to 2012. All the tissue samples were taken from the lungs and immediately frozen at −80 °C.

All 5 PRRSV-positive and 5 PRRSV-negative samples were 4-week-old, TTSuV1-positive pigs, obtained from a PRRSV-eradicating farm. All the piglets were antigen-negative for swine influenza virus, pseudorabies virus, classical swine fever virus, PCV2 and porcine parvovirus by PCR (or RT-PCR) method. All piglets were euthanized for collection of blood samples, heart, liver, spleen, lung, kidney, pancreas, trachea, fat, intestine, MLNs, ILNs, ASs, NSs, ovary or testis samples. As tissue samples had residual blood, all samples were washed three times with DEPC water (except serum, ASs and NSs), and all samples were stored at −20 °C until analyzed.

2.2. Viral nucleic acid extraction

Viral nucleic acid was extracted from 200 μL tissue homogenate (10%, w/v) or from ~25-mg tissue samples using the Virus DNA/RNA Extraction Kit II (Geneaid, USA) according to the manufacturer's instructions. Extracted DNA/RNA was eluted twice with 25 μL sterile distilled water to give a final extract volume of 50 μL. All DNA/RNA extraction procedures included a negative control, containing only PBS as extraction substrate.

2.3. Detection of PRRSV and TTSuV1

PRRSV RNA was assessed by RT-PCR using a broadly reactive primer pair, AU1/AU2 which amplified a 433-bp fragment of American type PRRSV following the reaction conditions previously described (Mardassi et al., 1994). Identification of TTSuV1 infection was done by PCR as described previously (Zhang et al., 2012).

2.4. Real-time PCR for detecting TTSuV1 titers in tissues

In all the samples, TTSuV1 DNA was detected by real-time PCR as previously described (Brassard et al., 2010; Segales et al., 2009). TaqMan PCR assays were performed in 25 μL reaction mixture comprising 2.5 μL extracted DNA and 22.5 μL master mix, which was made with the Brilliant I QPCR Core Reagent Kit (Stratagene) and contained 5.0 mmol/L $MgCl_2$, 300 nmol/L QCOM forward primer, 150 nmol/L QCOM reverse primer, and 300 nmol/L TaqMan probe. PCR amplification was performed with a Stratagene Mx 3005p system (Brassard et al., 2010). Student's t test was used to compare viral loads.

3. Results and discussion

Out of 2384 samples, 1115 and 1361 were positive for PRRSV and TTSuV1, respectively. From the geographical distribution, PRRSV and TTSuV1 were detected from all 6 provinces, the prevalence rate was very high. This indicates that the two viruses are widely distributed in China, and it might reach the distribution equilibrium to some extent. TTSuV1 detection rate in PRRSV-positive samples was >60% in 4 provinces: Jiangxi was the highest (69.5%) and Shanghai the lowest (51.9%). In PRRSV-negative samples, the rate was >50% in 3 provinces: Jiangxi was the highest (55.1%) and Shanghai the lowest (33.6%). Among these, the overall prevalence of TTSuV1 in PRRSV positive/negative samples was 65.3% and 47.2%, respectively, and the former rate was higher than the latter. Table 1 shows that except Shandong province, the phenomenon of the prevalence rate of TTSuV1 in PRRSV-positive was higher than negative existed in each province. The results indicate that PRRSV and TTSuV1 are widely distributed in swine in China and TTSuV1 is mostly likely to have a synergistic effect with PRRSV.

The genomic DNA was extracted from blood samples, heart, liver, spleen, lung, kidney, pancreas, trachea, fat, intestine, MLNs, ILNs, ASs, NSs, ovary and testis samples, aseptically collected from 5 PRRSV-negative piglets. The viral load of TTSuV1 was detected by real-time PCR (Table 2). In the PRRSV-negative group, the content of

Table 1
Detection of TTSuV1 in PRRSV-positive/negative samples.

Province	PRRSV positive		PRRSV negative	
	Samples	TTSuV1	Samples	TTSuV1
Jiangsu	316	249 (67.7) ^{aA}	356	182 (51.1) ^{bB}
Anhui	342	235 (68.7) ^{aA}	335	178 (53.1) ^{bB}
Zhejiang	207	128 (61.8) ^{aA}	216	96 (44.4) ^{bB}
Shandong	91	53 (58.2) ^{aA}	180	67 (37.2) ^{bB}
Shanghai	77	40 (51.9) ^{aA}	113	38 (33.6) ^{bB}
Jiangxi	82	57 (69.5)	69	38 (55.1)
Total	1115	762 (65.3) ^{aA}	1269	599 (47.2) ^{bB}

Data with different capital letter superscripts within the same row differ significantly ($p < 0.01$). Data with different small letter superscripts within the same row differ significantly ($p < 0.05$).