

# Molecular Detection and Genomic Characterization of Torque teno sus virus 1 in Jiangnan Area of China

Guangling Tian Houjun He, Weisong Wang

College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang 330045, China

**Abstract-** In this study, we detected Torque teno sus virus 1(TTSuV 1) in blood samples obtained from pigs in Jiangnan area of China, also characterized complete genomes of TTSuV 1. Genomes of TTSuV1 were obtained to examine the diversity and evolution of swine TTVs. The results suggest that JX TTSuV1 strain have a different sequence identity between 67.7% to 97.7% , and shared the highest identity (97.7%) with the Japan strain (AB076001).

**Index Terms-** Torque teno sus virus, Complete genome, Sequence analysis, Pig

## I. INTRODUCTION

Torque teno viruses (TTVs) are small, circular, non-enveloped viruses, which has been classified as a member of the discovered *Anelloviridae* family<sup>[i,ii]</sup>. TTVs were first found in a Japanese patient with posttransfusion hepatitis and then have also been found in other species including cats, dogs, pigs, cows and sheep<sup>[iii,iv]</sup>. There are two distinct genogroups have been identified as Torque teno sus virus 1(TTSuV1) and Torque teno sus virus 2 (TTSuV2) with wide spread in swine populations. TTSuVs have been shown to be involved in co-infection with other disease such as porcine dermatitis and nephropathy syndrome(PDNS) and postweaning multisystemic wasting syndrome(PMWS). Data suggests that TTSuVs were associated with some pathological conditions of swine<sup>[v]</sup>.

The diversity and phylogeny of this virus has not been fully studied because of low number of swine TTSuV complete genome sequences. The transcriptional profiles and genomic organization of viruses are similar with high genetic divergence. The viral genome length varies from 2.0 kb to 3.9 kb depending on the host species<sup>[vi]</sup>. The genome of swine TTV is approximately 2.8 kb and our aim was to characterize complete genomes of TTSuV 1 and to examine the diversity and evolution of swine TTVs<sup>[vii]</sup>.

## II. MATERIALS AND METHODS

### Sample preparation

The sera samples obtained from farm in Jiangnan area of China were preserved in our lab under -80°C. Viral DNA was extracted using magnetic genomic DNA extraction kit(TianGen) and desolved under -20°C. The full-length genomes of TTSuV1 were amplified with proofreading DNA polymerase (TaKaRa LA Taq, TaKaRa Bio Inc, Japan) using specific primers located in the UTR.

### Primer design

**Table1 TTSuV1 complete genome amplification primers**

Primers	Sequences of primers	Size
Forward	5'-	2878
	GGCGGACCTGATTGAA	
Reverse	GACTGAAAACCGTT-3'	bp
	5'-	
	GGCAGCAAACGTGGTG	
	CGAGCGAAGCGA-3'	

The complete genome sequences of TTSuV1 was found from the GenBank and aligned using the software program DNASTar (DNASTAR, Inc. Madison, WI). A pair of primers to detect TTSuV1 was designed using the Primer Premier Software (version 5.0) based on conserved sequences of TTSuV1. Another pair of primers to amplification was also designed and this was showed in Table 1.

### Detection of TTSuV1

SN-PCR was used to detect TTSuV1. PCR reaction mixture contains 2.5μL template DNA, 12μL rTaq (TakaRa Bio Inc, Japan), 1μL of each primer (25μL), and ddH<sub>2</sub>O to a total volume of 25μL. The reaction was performed by preheating for 5 min at 95°C, followed by 35 cycles at 94°C for 30s, at 54°C (the second reaction is 52°C) for 30s, and at 72°C for 40s, and a final extension for 10min at 72°C. PCR product (5μL) was analyzed using electrophoresis on a 1% (w/v) agarose gel and visualized with JS-650D equipment (PeiQing, China). Then agarose gel recycling was carried on to obtain the target strip and the product of recycling was also detected using electrophoresis. The genomic DNA was analyzed by agarose gel electrophoresis and PCR amplification was carried out for further comparison.

### PCR amplification

Positive sera samples detected by PCR were selected for amplification of complete genomes of TTSuV1. Complete genomes of TTSuV1 was amplified by PCR using specific primers showed as Table 1 and LA Taq DNA polymerase (TakaRa Bio Inc, Japan) according to TaKaRa LA Taq instructions. The amplification primers were amplified according to sequence in Genbank(Accession number: AB076001). Reaction system containing 10×LA buffer, dNTP Mixture 2.5Mm, LA rTaq, 1.25 U, primer 10 pmol/μL, DNA template 2.5μL was diluted with double-distilled water(DDW) in total volume of 25μL. The amplification procedure was as followed: 5min at 95°C, 30s at 94°C, 30s at 59°C, 3min at 72°C, followed by

35 cycles of 30 s at 94°C and a final extension for 10min at 72°C. The amplified positive products were purified with a TaKaRa MiniBEST Agrose Gel DNA Extraction Kit (Ver.4.0) and cloned into the pMD18-T Vector. The cloned products were then sequenced using an ABI 377 DNA sequencer (ABI, USA).

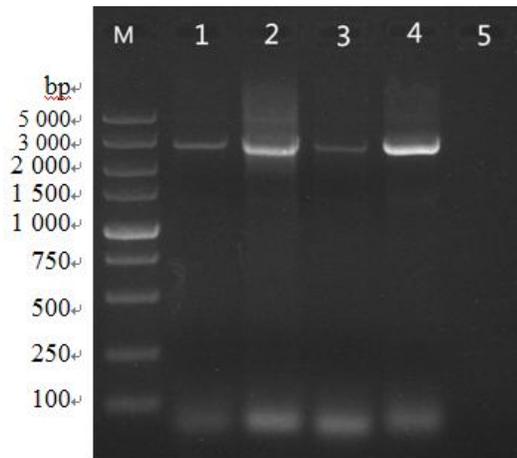
**Sequence analysis**

Complete genomic sequences of TTSuVs strains were obtained from GenBank database. Phylogenetic analyses were performed using the neighbor joining method in MEGA 5.1. Phylogenetic tree was also constructed based on the ORF1 sequences of strains.

**III. RESULTS AND DISCUSSION**

**PCR amplification**

TTSuV was detected in pigs from all the different districts using SN-PCR. The positive product of TTSuV1 was chosen to be amplified. The amplified PCR products, positive stripes of amplification, were observed to be 2878bp of TTSuV1 using agarose gel electrophoresis. The results were shown as Fig.1.



**Fig1. PCR amplification of Tc TSuV1 complete genome. M: DL 5 000 Marker; 1~4 : Production of PCR Amplification ; 5:Negative control: M: DL 2000 DNA Maker.**

**Sequence analysis**

TTSuV1 amplified from swine sera were sequenced and characterized together with several already known genomic sequences. The sequencing results were analyzed in comparison using Chroms and DNASTar biological software. Complete genome sequence with 2893bp length of TTSuV1 was obtained and made homology analysis together with 9 strains from China and 5 strains from Spain, America, Canada, Japan and Brazil. The map of homology comparison of the TTSuV1 complete genome was obtained using Lasergene software was shown in Fig.2.

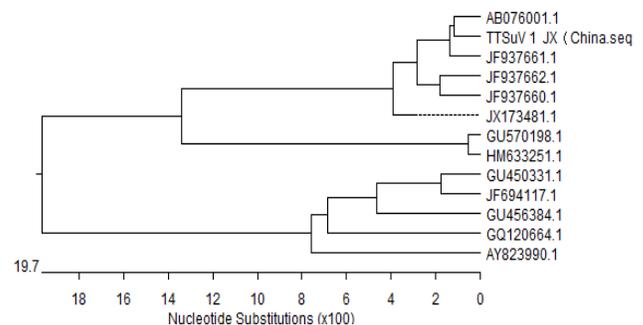
1	88.2	95.5	69.4	69.7	69.7	76.4	78.9	68.4	94.6	95.0	95.6	95.1	1	JX173481.1
2	40.9	68.7	86.2	85.5	86.2	69.4	71.0	83.3	67.8	67.8	67.4	68.3	2	AY823990.1
3	4.6	40.0	69.3	69.6	69.6	75.9	78.5	68.1	94.6	97.6	94.8	97.7	3	AB076001.1
4	39.3	14.8	39.5	87.2	88.4	68.4	71.0	84.4	68.3	68.4	68.3	68.8	4	GQ120664.1
5	38.9	15.6	39.1	14.1	91.8	68.0	70.6	94.6	69.0	69.0	69.2	69.2	5	GU450331.1
6	39.0	14.7	39.1	12.7	8.7	68.5	71.0	88.9	68.6	68.7	68.8	69.0	6	GU456384.1
7	25.5	37.2	26.3	38.1	38.7	38.0	96.6	70.5	76.6	75.4	76.8	75.9	7	GU570198.1
8	24.8	36.1	25.4	36.7	37.4	36.8	1.0	69.9	78.6	77.9	78.7	78.3	8	HM633251.1
9	38.7	17.5	39.3	15.4	3.5	9.9	36.8	36.2	68.0	67.3	68.2	67.7	9	JF694117.1
10	5.7	41.6	5.6	41.3	40.1	40.9	25.5	25.3	39.7	94.8	96.5	94.4	10	JF937660.1
11	5.2	41.6	2.5	41.1	40.2	40.7	27.0	26.2	40.8	5.4	94.6	97.2	11	JF937661.1
12	4.5	42.2	5.4	41.3	39.8	40.4	25.2	25.1	39.2	3.6	5.6	94.6	12	JF937662.1
13	5.1	40.8	2.3	40.3	39.8	40.1	26.3	25.7	40.1	5.8	2.8	5.5	13	JX TTSuV-1 (China.)

**Fig 2. Homology comparison of theTTSuV1 isolated complete genome.**The upper-left triangle date represent divergence of complete genomic nucleotide sequence, but the upper-right triangle date represent identity of the complete genomic nucleotide sequence of TTSuV 1.

According to homology analysis, nucleic acid sequence homology was 67.7%~97.7% between the TTSuV1 in this study and TTSuVs from GenBank. The TTSuV1 sequences in this study showed a sequence similarity of 97.7% to strain AB076001 from Japan and 97.2% to strain GU937661 from Fujian in Chia. Sequence similarity of TTSuV1 was low compared with strain AY823990 from Brazil and JF694117 from Sichuan in Chian. Conserved region of TTSuV1 was located in 1nt~706nt and 2 276nt~2 893nt. ORF1was in 534nt ~2 498nt. ORF2 was in 430nt~651nt and ORF3 was in 430nt~647nt and 2 078nt~2 498nt.

**Phylogenetic Analysis**

The sequences from the PCR products of TTSuV1 positive samples were used for diversity and phylogenetic studies according to the complete genetic sequences of TTSuV1 from NCBI. The TTSuV1 sequences in this study showed a low sequence similarity of 50% to TTSuV2. The identity ranged from 90 to 98% compared with TTSuV1 sequences originated from other places of the world. This TTSuV1 sequences had a higher sequence divergence with these chosen strains between 66.7-97.7%. The phylogenetic tree with two main clades was showed in Fig.3.



**Fig 3. Phylogenetic tree derived from the 16S rRNA gene sequences showing relationships of TTSuV1 to all described species.** Numbers at nodes indicate bootstrap values(percentage of 1000 replicates). Bar value estimated nucleotide substitutions per site.

Reports from many parts of the world suggest that TTSuV1 is widespread among pig populations<sup>[viii,ix]</sup>. In recent years,

TTSuVs and PCV2 have high rate in co-infection with subclinical symptoms but not result in associated diseases<sup>[x,xi]</sup>. The results of Blast comparative analysis suggested that TTSuV 1 in this study has 79%~98% of nucleotide similarity with complete genome sequences of TTSuV 1 from NCBI. The coverage rate of HM633252, HM633242 and JX535325 was 100% comparative with TTSuV 1 in this study. AB076001 from Japan has highest genetic relationship with TTSuV1, so we can make it as reference in study of epidemic disease.

#### IV. CONCLUSIONS

In conclusion, this study is the first in assessing the presence of TTSuV1 in domestic pigs in Jiangnan area of China. In this limited study we have suggested that, like in other places of the world, TTSuV1 is widespread among domestic pigs in China.

#### ACKNOWLEDGEMENTS

This work was supported by the Jiangxi Agricultural University and was supported by grants from Nanchang education bureau.

#### REFERENCES

- [1] Krakowka S, Ellis J A. Evaluation of the effects of porcine genogroup 1 torque teno virus in gnotobiotic swine. *Am J Vet Res*. 2008, 69: 1623-1629.
- [2] Nishizawa T, Okamoto H, Konishi K, et al. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology [J]. *Biochem Biophys Res Commun*, 1997, 241(1): 92-97
- [3] Cortey M, Macera L, Segalés J, et al. Genetic variability and phylogeny of Torque teno sus virus 1 (TTSuV1) and 2 (TTSuV2) based on complete genomes [J]. *Veterinary microbiology*, 2011, 148(2): 125-131.
- [4] Biagini P, Gallian P, Attoui H, et al. Genetic analysis of full-length genomes and subgenomic sequences of TT virus-like mini viruses human isolates [J]. *J Gen Virol*, 2001, 82 : 379-383.

- [5] P. Biagini. Classification of TTV and Related Viruses ( anelloviruses ) [J]. *Curr Top Microbiol Immunol*, 2009; 331:21-33.
- [6] Hohne M, Berg T, Muller A R, et al. Detection of sequences of TT virus, a novel DNA virus, in German patients [J]. *J Gen Virol*, 1998, 79: 2761-2764.
- [7] Krakowka S, Hartunian C, Hamberg A, et al. Evaluation of induction of porcine dermatitis and nephropathy syndrome in gnotobiotic pigs with negative results for porcine circovirus type 2 [J]. *American journal of veterinary research*, 2008, 69(12): 1615-1622.
- [8] Ellis J A, Allan G, Krakowka S. Effect of coinfection with genogroup 1 porcine torque teno virus on porcine circovirus type 2-associated postweaning multisystemic wasting syndrome in gnotobiotic pigs [J]. *American journal of veterinary research*, 2008, 69(12): 1608-1614.
- [9] Okamoto H, Takahashi M, Nishizawa T, et al. Genomic characterization of TT viruses (TTVs) in Pigs, cats and dogs and their relatedness with species-specific TTVs in Primates and tupaia [J]. *J Gen Virol*, 2002, 83(6): 1291-1297.
- [10] Taira O, Ogawa H, Nagao A, K, et al. Prevalence of swine Torque teno virus genogroups 1 and 2 in Japanese swine with suspected postweaning multisystemic wasting syndrome and porcine respiratory disease complex [J]. *Vet. Microbiol*, 2009, 139(3-4): 347-350.
- [11] Gallei A, Pesch S, Esling W S, et al. Porcine Torque teno virus: determination of viral genomic loads by genogroup-specific multiplex RT-PCR, detection of frequent multiple infections with genogroups 1 or 2, and establishment of viral full-length sequences [J]. *Vet. Microbiol*, 2010, 143(2-4): 202-212.

#### AUTHORS

**First Author** – Guangling Tian, MD, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China.

**Second Author** – Houjun He, PhD, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China.

**Third Author** – Weisong Wang, MD, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China.

**Fourth Author** – Houjun He, PhD, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang, China.