Polish Journal of Veterinary Sciences Vol. 24, No. 2 (2021), 307–312

DOI 10.24425/pjvs.2021.137667

Short communication

Proposed genotype definition of *Porcine sapelovirus*

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Abstract

Conventionally, *Porcine sapelovirus* (PSV) has been considered to comprise a single genotype, PSV-1; however, a potentially novel member of PSV was recently discovered. In the present study, we propose a genotype definition of PSV based on phylogenetic and genetic analyses of the polyprotein, *P1,* and *VP1* genes of available PSV sequences. Two genotypes, with proposed names PSV-1 and PSV-2, were identified. Moreover, the cut-off values (number of differences per site between amino acid sequences) for the definition of genotypes were established to be 0.1115 (polyprotein), 0.176 (*P1*), and 0.272 (*VP1*). The findings of this study are expected to enrich knowledge of PSV classification.

Key words: *Porcine sapelovirus*, genotype, phylogenetic and genetic analyses

Introduction

Porcine sapelovirus (PSV) is recognized as the infectious agent of polioencephalomyelitis, which is considered to have a severe impact on the health of swine (Arruda et al. 2017). The clinical signs of PSV infections have been associated with series typical neurological symptoms (including the spinal cord damage, recumbency, ataxia, and paraparesis), diarrhea, and respiratory distress (Lan et al. 2011).

PSV, taxonomically designated *Sapelovirus A*, belongs to the genus *Sapelovirus* within the family *Picornaviridae* (Zell et al. 2017). In the 10th report of the International Committee on Taxonomy of Viruses (ICTV), two species were recorded for the genus *Sapelovirus*: *Sapelovirus A* and *Sapelovirus B*. *Sapelovirus A* has only a single type (PSV-1) (Zell et al. 2017). Recent developments in viral metagenomics led to the discovery of a potentially novel genotype of PSV in Hungary (Boros et al. 2020). Thus, this new discovery highlights the need for further exploration of the definition of genotypes in PSV. Accordingly, the objective of the present study was to establish a genotype definition for PSV based on phylogenetic and genetic analyses.

Materials and Methods

PSV BLAST was used to identify closely related sequences in the GenBank database (up to July 2020). Identical sequences were eliminated after alignment using the mafft program. Nucleotide sequences for

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Table 1. Sapelovirus strains used in the study.

Virus isolate	Collection date	GenBank accession No.	Geographic origin	Sequence length
1	2	3	4	5
HuN1	2015	KX354740	China	6996
HuN2	2015	KX354741	China	6996
HuN29	2017	MF440657	China	7220
HuN30	2017	MF440658	China	7259
HuN20	2017	MF440648	China	7223
HuN27	2017	MF440655	China	7263
HuN13	2016	MF440641	China	7254
HuN14	2016	MF440642	China	7152
HuN11	2016	MF440639	China	7231
HuN16	2016	MF440644	China	7227
HuN8	2016	MF440636	China	7222
HuN7	2016	MF440635	China	7264
HuN18	2016	MF440646	China	7225
HuN5	2016	MF440633	China	7218
HuN24	2017	MF440652	China	7221
HuN15	2016	MF440643	China	7221
QT2013	2013	KJ463384	China	7532
HuN33	2014	MF440661	China	7222
NM02_C1	2017	MK378953	China	7458
HuN31	2017	MF440659	China	7232
HuN6	2016	MF440634	China	7210
HeB04	2017	MK378925	China	7382
XTND/2018	2018	LC493088	Viet Nam	7537
HuN25	2014	MF440653	China	7254
YN02	2017	MK378966	China	7347
csh	2009	HQ875059	China	7502
HuN4	2015	KX354743	China	6999
JD2011	2011	KF539414	China	7572
YC2011	2011	JX286666	China	7572
PSV-20-V	2018	LC508226	Zambia	7495
HuN23	2017	MF440651	China	7221
HuN3	2015	KX354742	China	6996
JXXY-C	2017	MH626635	China	7575
PSV/Porcine/JPN/MoI2/2016	2016	LC425414	Japan	7514
SHCM2019	2019	MN685785	China	7567
HuN32	2017	MF440660	China	7227
PSV/Porcine/JPN/HgOg11/2018	2018	LC425417	Japan	7521
PSV-23-V	2018	LC508230	Zambia	7462
PSV/Porcine/JPN/MoI3/2016	2016	LC425415	Japan	7540
PSV/Porcine/JPN/MoI2-2/2015	2015	LC425394	Japan	7425
PSV-26-B	2018	LC508232	Zambia	7493
PSV/Porcine/JPN/HgTa2-2/2015	2015	LC425404	Japan	7525

Cont. Table 1.

1	2	3	4	5
PSV/Porcine/JPN/HgOg2-5/2015	2015	LC425395	Japan	7480
HuN12	2016	MF440640	China	7227
HuN9	2016	MF440637	China	7245
PSV/Porcine/JPN/HgYa1/2016	2016	LC425413	Japan	7526
PSV/Porcine/JPN/Iba26-489S/2015	2014	LC425397	Japan	7531
KS055217	2005	KJ821021	South Korea	7542
KS05151	2005	KJ821020	South Korea	7566
PSV/Porcine/JPN/HkKa2-3/2015	2015	LC425403	Japan	7540
KS04105	2004	KJ821019	South Korea	7542
PSV-46-V	2018	LC508233	Zambia	7469
PSV/Porcine/JPN/HgYa2-3/2015	2015	LC425407	Japan	7535
PSV/Porcine/JPN/HgTa2-1/2015	2015	LC425401	Japan	7540
USA/IA33375/2015	-	KX574284	USA	7565
PSV/Porcine/JPN/HgYa2-1/2015	2015	LC425405	Japan	7525
PSV/Porcine/JPN/DeTk2-2/2015	2015	LC425396	Japan	7428
PSV/Porcine/JPN/Ishi-Ka2/2015	2015	LC425398	Japan	7543
Jpsv447	2009	LC326556	Japan	7582
PSV/Porcine/JPN/Ishi-Ka2/2017	2017	LC425416	Japan	7534
PSV/Porcine/JPN/Ishi-Im3/2015	2015	LC425410	Japan	7554
PSV/Porcine/JPN/Ishi-Miya3/2015	2015	LC425399	Japan	7508
ISU-SHIC	2016	KX810827	USA	7389
PSV/Porcine/JPN/Ishi-Ya8/2015	2015	LC425412	Japan	7528
IVRI/PSV/SPF/C-6/2015	2015	KY053835	India	7491
V13	-	AF406813	Germany	7491
OPY-1-Corsica-2017	2017	MH513612	France	7532
DIAPD5469-10	2015	MK497044	Italy	7564
PSV_GER_L00798-K11_14-02_2014	2014	LT900497	Germany	7499
SZ1M-F/PSV/HUN/2013	2013	MN807752	Hungary	7534
SwPSV75BO2012	2012	MN836683	Italy	7560
GX03_C1	2017	MK378906	China	7263
GX04_C1	2017	MK378907	China	7300
HLJ01_C1	2017	MK378928	China	6445
GZ04_C1	2017	MK378918	China	6829
ZJ01_C1	2017	MK378967	China	7133
AH01_C1	2017	MK378881	China	7191
GX01_C1	2017	MK378899	China	4967
Sek_1562/98	1998	AY392556	Germany	3968
Po_5116	1998	AY392538	Germany	3969
26-T-XII	1998	AY392544	Germany	3688
16-S-X	1998	AY392543	Germany	3699
EF9-F/PSV/HUN/2016	2016	MN807773	Hungary	854
JS/CHN/2016	2016	MH422121	China	855
SD/CHN/2016	2015	MH422124	China	855
NMG02/CHN/2016	2016	MH422125	China	855

Cont. Table 1.

1	2	3	4	5
A5-RS/PSV/HUN/2016	2016	MN807776	Hungary	879
A1-RS/PSV/HUN/2016	2016	MN807754	Hungary	879
GD1-RS/PSV/HUN/2016	2016	MN807768	Hungary	879
A3-RS/PSV/HUN/2016	2016	MN807756	Hungary	867
P1-3-3	2007	KF705632	Spain	1171
TM1121-F/PSV/HUN/2013	2013	MN807769	Hungary	879
SZ4M-F/PSV/HUN/2013	2013	MN807753	Hungary	867
B4-RS/PSV/HUN/2016	2016	MN807758	Hungary	879
BUV1-F/PSV/HUN/2013	2013	MN807759	Hungary	879
D4-NS/PSV/HUN/2016	2016	MN807764	Hungary	855
Gifu	-	AB619806	Japan	855
ZS2-F/PSV/HUN/2013	2013	MN807771	Hungary	855
EF2-F/PSV/HUN/2016	2016	MN807765	Hungary	879
VC4-EF2-F/PSV/HUN/2017	2017	MN807777	Hungary	879
HuN/CHN/2016	2016	MH422123	China	855
2383	-	AY064708	USA	8126
VRDL1	-	EU789367	USA	7971
WUHARV	2010	JX627573	USA	8059

- Not identified.

a total of 104 genomes were obtained, including 70 PSV (nearly) complete genomes and three SSV complete genomes, which were used as outgroup, as well as sequences of 12 *P1* and 19 *VP1* genes (Table 1).

Multiple alignments of deduced amino acid sequences of each sequence were carried out using the MUSCLE method within the MEGA 6.06 software package. For each dataset, a matrix of pairwise distances between sequences was constructed by the uncorrected p-distance method using MEGA 6.06 software. To determine possible cut-off values for distinguishing different PSV genotypes, a p-distance vs. frequency histogram was constructed using Excel software.

Phylogenetic analyses were carried out by IQ-TREE 1.6.12 using the maximum-likelihood (ML) method. The best-fit model of amino acids for each dataset was determined using ModelFinder. For the amino acid sequence analysis of polyprotein and *VP1* genes, the LG+R4 model was used for the ML method, whereas the LG+F+R6 model was used for the *P1* gene. Support for the estimated phylogeny was evaluated by a bootstrap analysis with 1000 replicates.

Results and Discussion

The p-distance vs. frequency histogram obtained from the sapelovirus sequences of 73 polyprotein, 85 *P1,* and 104 *VP1* genes clearly split into three districts (Fig. 1a, c, e). One district corresponded to the number of differences between the two species *Sapelovirus A* and *Sapelovirus B*, and the other two corresponded to differences within and between PSV genotypes. The p-distance among PSV genotypes for polyprotein, *P1,* and *VP1* genes ranged from 0.145 to 0.156 (mean 0.150), from 0.215 to 0.239 (mean 0.229), and from 0.304 to 0.376 (mean 0.353), respectively. These values were clearly higher than those obtained for the within-genotype analysis, which ranged from 0 to 0.078 (mean 0.042), from 0 to 0.137 (mean 0.072), and from 0 to 0.24 (mean 0.118) for polyprotein, *P1*, and *VP1* genes, respectively. The cut-off values for PSV genotype definition were finally established at 0.1115 (polyprotein), 0.176 (*P1*), and 0.272 (*VP1*), which is defined as the point with a fairly equidistant position between both partitions.

Moreover, the above definition of PSV genotypes matched our phylogenetic results (Fig. 1b, d, f). All three phylogenetic trees for polyprotein, *P1,* and *VP1* genes revealed a congruent topology of genotypes, and two genotypes were divided with high confidence values support. According to this definition, PSV includes two genotypes, tentatively named PSV-1 and PSV-2. Almost all PSV sequences were included within the PSV-1 branch, and only two PSV sequences, both recently found in Hungary, belonged to PSV-2.

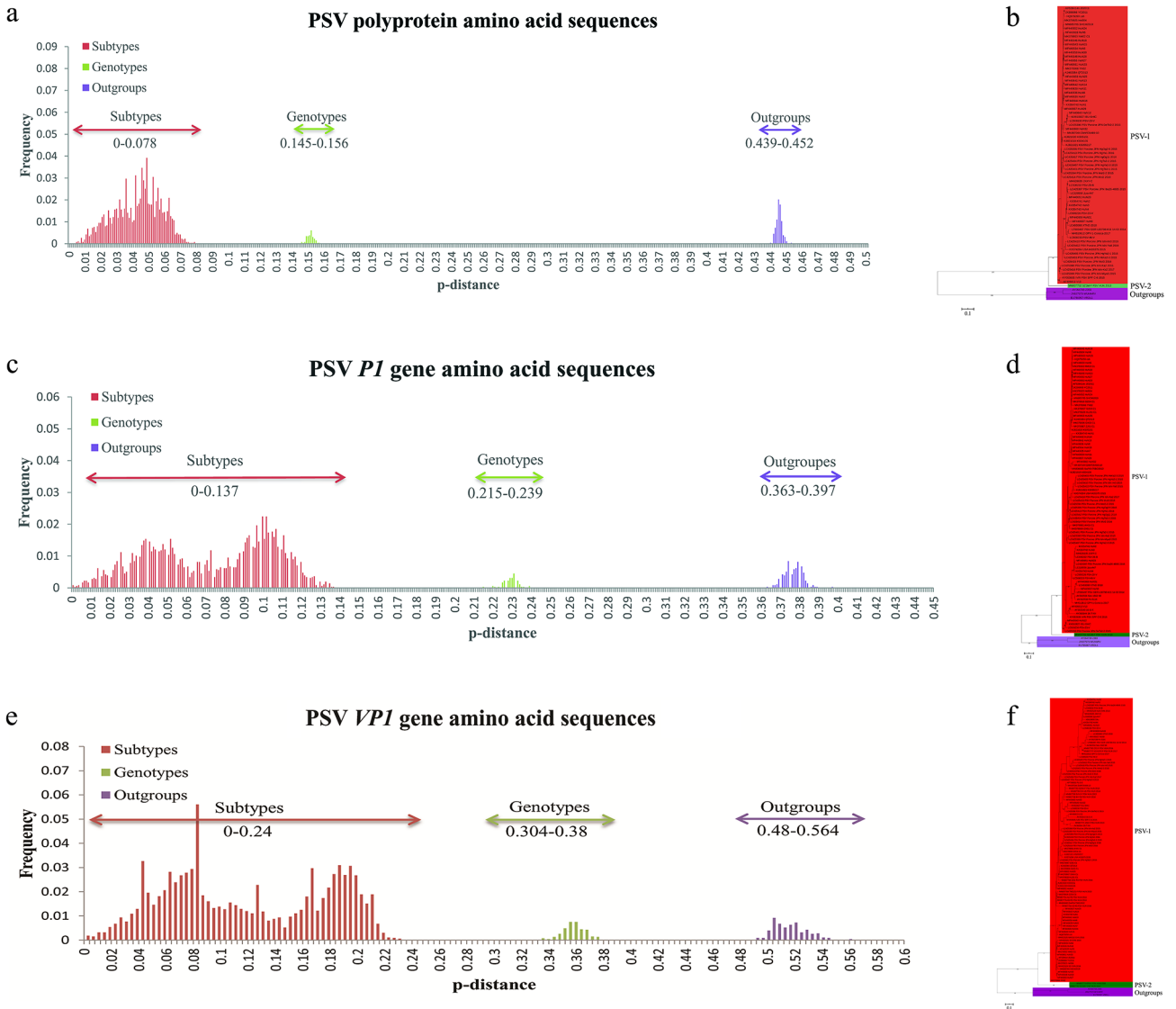

Fig 1. Frequency distribution of pairwise distances and evolutionary relationship among porcine sapeloviruses. The percentage of replicates in which the associated virus clustered together in the bootstrap test (1000 replicates) is shown next to the branches (only values > 70% are shown) in each tree. The scale bar indicates amino acid substitutions per site. *Sapelovirus B* strains of 2383, WUHARV, and VRDL1 were used as outgroups. (a) Frequency distribution of p-distance among polyprotein gene sequences of 70 available PSVs. (b) Evolutionary tree based on the amino acid sequences of the polyprotein gene. (c) Frequency distribution of p-distance among *P1* gene sequences of 82 available PSVs. (d) Evolutionary tree based on the amino acid sequences of the *P1* gene. (e) Frequency distribution of p-distance among *VP1* gene sequences of 101 available PSVs. (f) Evolutionary tree based on the amino acid sequences of the *VP1* gene.

Genotyping has been widely used for virus classification (Zhang et al. 2013) as a critical tool for surveillance of virus molecular epidemiology. However, the mandate of the ICTV only includes species demarcation criteria for the genus *Sapelovirus*, and does not consider classification below the species level. The capsids (encoded by *P1*) of picornaviruses are the main neutralization epitopes responsible for immunodominant differentiation (Racaniello 2001). Phylogenetic and genetic analysis based on the *P1* gene has been considered to be a reliable approach for other picornavirus genotyping (Yang et al. 2020). In the present study, phylogenetic and genetic analyses based on the *P1* gene revealed that PSV includes two distinct genotypes. This finding was supported by genetic evolutionary analyses based on the *VP1* and polyprotein sequences, suggesting that these genes are also reliable for PSV genotyping.

Originally, PSV, as a distinct serotype, was assigned to porcine enterovirus (PEV) 8 (Krumbholz et al. 2002). To date, there have been no reports describing the existence of another novel serotype among all currently known PSV strains. However, we found high amino acid sequence diversity (0.215-0.239) between PSV-1 and PSV-2 based on analysis of the *P1* gene, which is responsible for PSV neutralization epitopes. This

finding suggests that PSV-2 might represent a novel PSV serotype. Unfortunately, no PSV-2 strains have yet been isolated; thus, further studies are required to confirm their serotype.

Acknowledgements

This work was supported by the "Xiujiang" Talent Development Program of Yichun, China (grant 2019-11) and the National Natural Science Foundation of China (31960697).

References

Arruda PHE, Arruda BL, Schwartz KJ, Vannucci F, Resende T, Rovira A, Sundberg P, Nietfeld J, Hause BM (**2017**) Detection of a novel sapelovirus in central nervous tissue of pigs with polioencephalomyelitis in the USA. Transbound Emerg Dis 64: 311-315.

Boros Á, László Z, Pankovics P, Marosi A, Albert M, Cságola A, Bíró H, Fahsbender E, Delwart E, Reuter G (**2020**) High prevalence, genetic diversity and a potentially novel genotype of Sapelovirus A (Picornaviridae) in enteric and respiratory samples in Hungarian swine farms. J Gen Virol 101: 609-621.

Krumbholz A, Dauber M, Henke A, Birch-Hirschfeld E, Knowles NJ, Stelzner A, Zell R (**2002**) Sequencing of porcine enterovirus groups II and III reveals unique features of both virus groups. J Virol 76: 5813-5821.

Lan D, Ji W, Yang S, Cui L, Yang Z, Yuan C, Hua X (**2011**) Isolation and characterization of the first Chinese porcine sapelovirus strain. Arch Virol 156: 1567-1574.

Racaniello VR (**2001**) Picornaviridae: the viruses and their replication. In: Knipe DM, Howley PM (eds) Fields virology. Lippincott Williams & Wilkins, Philadelphia, pp 455-457.

Yang T, Lu Y, Zhang L, Li X (**2020**) Identification of novel genotypes belonging to the species Teschovirus A from indigenous pigs in western Jiangxi, China. Arch Virol 165: 993-1001.

Zell R, Delwart E, Gorbalenya AE, Hovi T, King AMQ, Knowles NJ, Lindberg AM, Pallansch MA, Palmenberg AC, Reuter G, Simmonds P, Skern T, Stanway G, Yamashita T, Ictv Report Consortium (**2017**) ICTV Virus Taxonomy Profile: Picornaviridae. J Gen Virol 98: 2421-2422.

Zhang Z, Jia R, Lu Y, Wang M, Zhu D, Chen S, Yin Z, Chen X, Cheng A (**2013**) Identification, genotyping, and molecular evolution analysis of duck circovirus. Gene 529: 288-295.