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SHORT REPORT

An extra insertion of tandem repeat sequence in African swine fever virus, China, 2019

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Abstract

On 7 March 2019, African swine fever in a domestic pig farm was detected in Guangxi Province of China. The phylogenetic analysis showed that its causative strain contained two tandem repeat sequence insertions in the intergenic region between the I73R and the I329L genes, and was different from previously reported strains in China and other countries.

Keywords African swine fever · China · Tandem repeat sequence

African swine fever (ASF) is one of the most important and dangerous viral diseases of pigs, which is caused by African swine fever virus (ASFV), a large and enveloped double-stranded DNA virus, belonging to the Asfarviridae family, genus Asfivirus. ASF was first described in Kenya in 1921 and spread outside Africa to the Iberian Peninsula in 1957 and to the Caribbean region in 1971. After national campaigns against ASF, most countries outside Africa eventually eradicated ASF (with the exception of Sardinia) in the late twentieth century. However, in 2007 ASFV re-entered Continental Europe due to a single incident in Georgia and continued to rapidly spread to neighboring countries [1]. In 2018, ASF spread into Hungary, China [2], Bulgaria, Belgium [3] and hit Mongolia, Vietnam [4], Cambodia, North Korea, Laos in the first half of 2019, and further struck Slovak Republic in July and Myanmar in August.

On 7 March 2019, a domestic pig farm with 3172 pigs was confirmed with ASFV infection by the China Animal Disease Control Center (CADC) of Guangxi Zhuang Autonomous Region. The location was approximately 300 km away from Vietnam (Fig. 1). On 12 March 2019, China Animal Health and Epidemiology Center (nominated as National African Swine Fever Reference laboratory) received five tissue samples (including spleen, inguinal lymph nodes, and mesenteric lymph nodes from two pigs) and performed the phylogenetic analysis and sequence alignment based on the comparison among the Sanger sequencing of B646L (encoding p72), E183L (encoding p54), CP204L (encoding p30), central variable region (CVR) of the ORF B602L, EP402R (encoding CD2-like protein) and tandem repeat sequences (TRS) in the intergenic region (IGR) between the I73R and I329L genes as previously described [2, 5].

The results of all the five tissue samples showed that the five sequencing genes (B646L, E183L, CP204L, B602L, and EP402R) shared 100% sequence identity with the current strains in China, which represent that the causative strain (China/Guangxi/2019/domestic pig) belonged to the P72 genotype II and CD2v serogroup 8 (Fig. 2). However, the China/Guangxi/2019/domestic pig contained two TRS insertions, and was different from the previously reported strains in China and other countries (Table 1).

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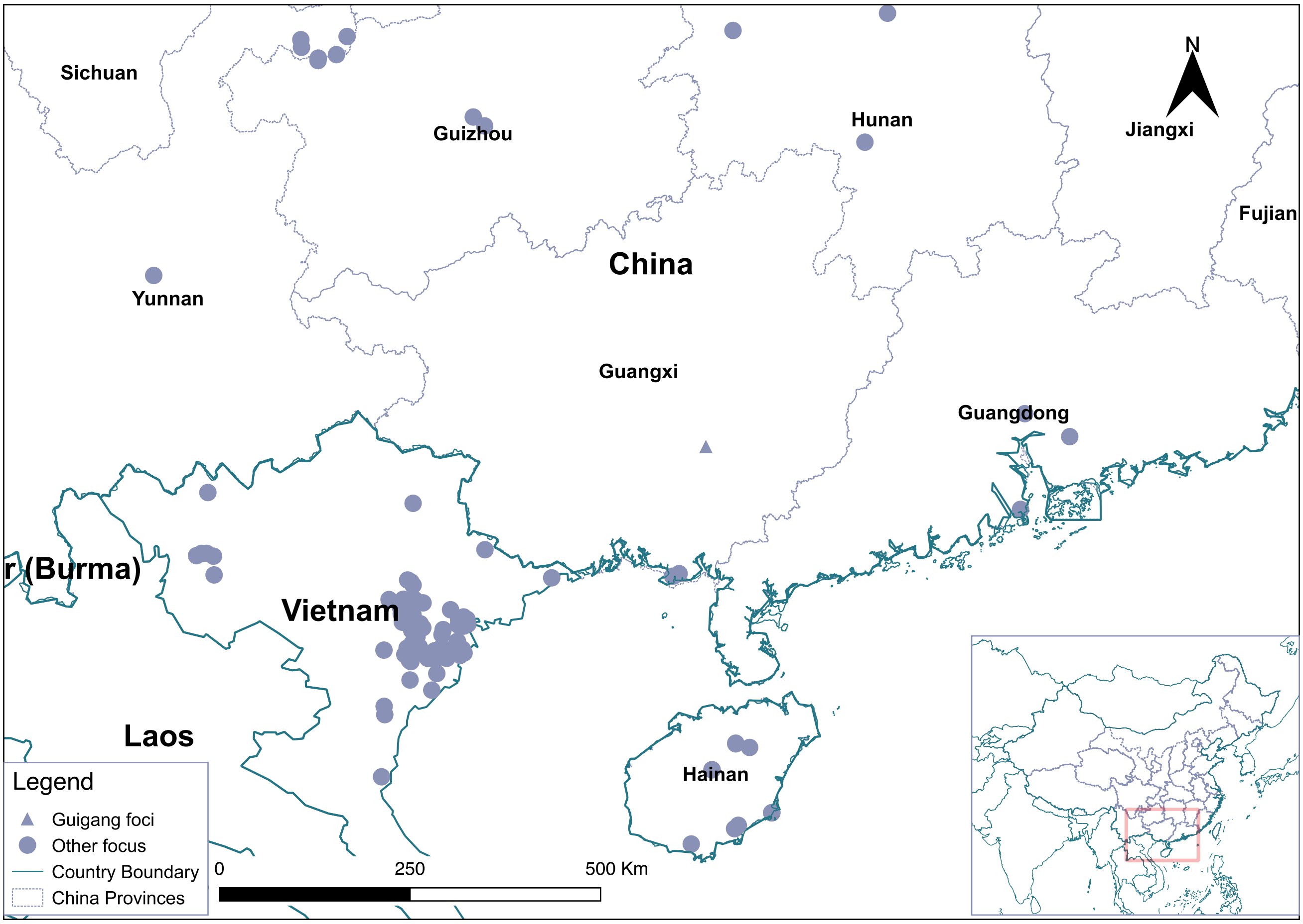

Fig. 1 African swine fever outbreaks around Guangxi Province since August 2018. The blue triangle indicates the location of the described outbreak in Guangxi Province

The existence of TRS insertions in IGR between the I73R and I329L genes was first described in 1992 [6], and subsequently analyzed as an effective ASFV genome marker to discriminate closely circulating ASFVs from Eastern Europe [7], Russia [8], and Italy [9]. Recent ASF outbreaks in China were caused by ASFVs of the IGR I variant (without extra TRS insertions in the IGR, only one case in wild boar) [10] and IGR II variant (has one TRS insertion in the IGR, circulating strain in domestic pig) [2]. The function and causation of two TRS insertions in the IGR (debut named as IGR III variant) are still unclear, but such genetic data are essential for tracing the source of ASFVs and extending our knowledge of virus evolution and epidemiology in China.

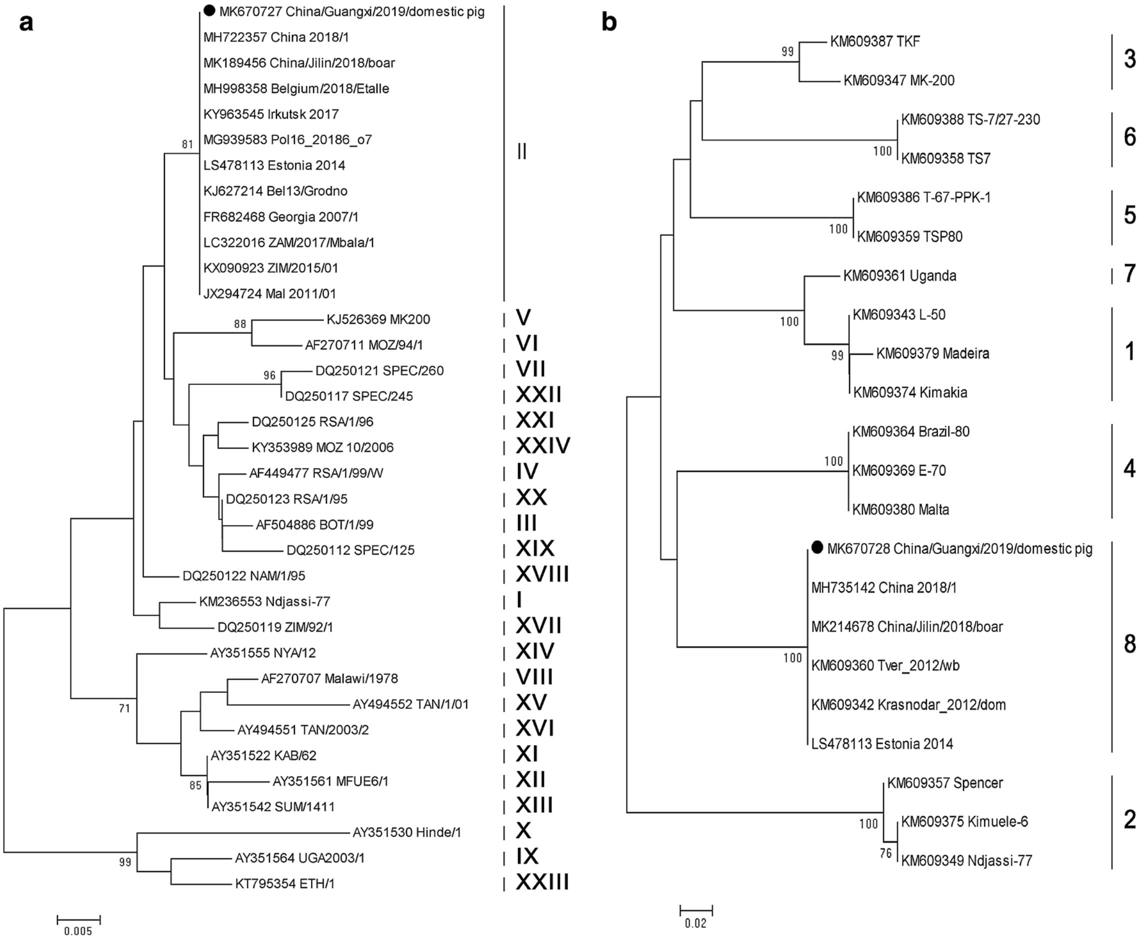

Fig. 2 Phylogenetic analysis of the African swine fever virus strain (China/Guangxi/2019/domestic pig) in Guangxi, China, 2019. **a** p72 genotype; **b** CD2v serogroup. The neighbor-joining method and Kimura 2-parameter model were used for the construction of phylogenetic trees in MEGA 5.0 software (https://www.megasoftware.net/). Numbers along branches indicate bootstrap values > 70% (1000 replicates). Black circles indicate causative virus from this study. Roman numerals to the right in panel **a** indicate p72 genotypes. Numbers to the right in panel **b** indicate CD2v serogroups. GenBank accession numbers are provided for all sequences. Scale bars indicate nucleotide substitutions per site

Table 1 Nucleotide sequence alignment of the partial intergenic region between I73R and I329L from African swine fever genotype II viruses, including domestic pig strain in China (China 2018/1; GenBank accession no. MH735144, and China/Guangxi/2019/domestic pig; GenBank accession no. MK670729) and wild boar strain in China (ASFV/Jilin/wb/2018; GenBank accession no. MK189457)

GenBank	Strain	Country	Year	Origin	Partial nucleotide sequence alignment of the TRS in the IGR between I73R and I329L
FR682468	Georgia 2007/1	Georgia	2007	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620028	Arm07	Armenia	2007	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620029	Abk07	Georgia	2007	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620030	Che07	Russia	2007	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MH910495	Georgia 2008/1	Georgia	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MH910496	Georgia 2008/2	Georgia	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620031	Ing08	Russia	2008	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620032	Oren08	Russia	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620033	NO08/Av	Russia	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620034	NO08/Ap	Russia	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620035	Az08D	Azerbaijan	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620036	Az08B	Azerbaijan	2008	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620048	Dagestan09	Russia	2009	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620049	Kalmykia09	Russia	2009	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620050	StPet09	Russia	2009	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620051	Rostov09	Russia	2009	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620038	Tver0511/Torjo	Russia	2011	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620039	Tver0812/Bolo	Russia	2012	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620040	Tver0712/Les	Russia	2012	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620041	Tver0312/Torjo	Russia	2012	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620042	Tver0312/Novo	Russia	2012	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137626	Tver06/2012	Russia	2012	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137627	Kashinskiy09/2012	Russia	2012	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137628	N.Novgorod_07/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137629	Moscow_07/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137632	Smolensk_06/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137630	Bryansk_05/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137631	Smolensk_05/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939584	Pol16_20538_o9	Poland	2016	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939586	Pol16_29413_o23	Poland	2016	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939587	Pol17_03029_C201	Poland	2017	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939589	Pol17_05838_C220	Poland	2017	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KY982843	Irkutsk2017	Russia	2017	Domestic pig	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
MK189457	China/Jilin/2018/boar	China	2018	Wild Boar	CAAGTATATAGGAATATATAGGAATATATA---------------------------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620037	Ukr12/Zapo	Ukraine	2012	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620043	Bel13/Grodno	Belarus	2013	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137633	Tula_08/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137634	Smolensk_09/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137635	Voroneg_07/13	Russia	2013	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137636	Belgorod_06/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137637	Volgograd_01/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137638	Bryansk_02/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137639	Kaluga_01/14	Russia	2014	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137640	Tula_02/14	Russia	2014	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137641	Tula_06/13	Russia	2013	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137642	Volgograd_02/14	Russia	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137643	Kaluga_08/14	Russia	2014	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KP137644	Tula_01/14	Russia	2014	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620044	Lt14/1490	Lithuania	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620045	Lt14/1482	Lithuania	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620046	Pol14/Sz	Poland	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KJ620047	Pol14/Krus	Poland	2014	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
LS478113	Estonia 2014	Estonia	2014	NA	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KY385895	Volgograd2015	Russia	2015	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KY385896	Bryansk2015	Russia	2015	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KY385893	Voronezh 2016	Russia	2016	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
KY385894	Lipetsk2016	Russia	2016	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939583	Pol16_20186_o7	Poland	2016	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939585	Pol16_20540_o10	Poland	2016	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MG939588	Pol17_04461_C210	Poland	2017	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MH998359	ASFV/Etalle/wb/2018	Belgium	2018	Wild Boar	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MH735144	China 2018/1	China	2018	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATA----------------GAAATATATAGAAATAGCTAAGCTTAATAC
MK670729	China/Guangxi/2019/domestic pig	China	2019	Domestic pig	CAAGTATATAGGAATATATAGGAATATATAGGAATATATAGGAATATATAGAAATATATAGAAATAGCTAAGCTTAATAC

Table 1 (continued)

The mutation that results in the insertion of additional TRS (GGAATATATA) in the ASFVs is indicated by gray shading. NA represents not available

Author contributions XW and ZW designed the study. SG, YL, QW, and WR performed the experiments and analyzed the data together with LL, JL, and JB. SG and YL wrote the initial draft of the manuscript and revised the manuscript together with JL, XW and ZW.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of China Animal Health and Epidemiology Center (No. 2019-12). All the applicable international, national, and institutional guidelines for the care and use of animals were followed.

References

1. Sanchez-Cordon PJ, Montoya M, Reis AL, Dixon LK (2018) African swine fever: a re-emerging viral disease threatening the global pig industry. Vet J 233:41–48
2. Ge S, Li J, Fan X, Liu F, Li L, Wang Q, Ren W, Bao J, Liu C, Wang H, Liu Y, Zhang Y, Xu T, Wu X (2018) Wang Z (2018) Molecular characterization of African swine fever virus, China. Emerg Infect Dis 24(11):2131–2133
3. Linden A, Licoppe A, Volpe R, Paternostre J, Lesenfants C, Cassart D, Garigliany M, Tignon M, van den Berg T, Desmecht D, Cay AB (2019) Summer 2018: African swine fever virus hits north-western Europe. Transbound Emerg Dis 66(1):54–55
4. Le VP, Jeong DG, Yoon SW, Kwon HM, Trinh TBN, Nguyen TL, Bui TTN, Oh J, Kim JB, Cheong KM, Van Tuyen N, Bae E, Vu TTH, Yeom M, Na W, Song D (2019) Outbreak of African swine fever, Vietnam. Emerg Infect Dis 25(7):1433–1435
5. Onzere CK, Bastos AD, Okoth EA, Lichoti JK, Bochere EN, Owido MG, Ndambuki G, Bronsvoort M, Bishop RP (2018) Multi-locus sequence typing of African swine fever viruses from endemic regions of Kenya and Eastern Uganda (2011–2013) reveals rapid B602L central variable region evolution. Virus Genes 54(1):111–123
6. Rodriguez JM, Salas ML, Vinuela E (1992) Genes homologous to ubiquitin-conjugating proteins and eukaryotic transcription factor SII in African swine fever virus. Virology 186(1):40–52
7. Gallardo C, Fernandez-Pinero J, Pelayo V, Gazaev I, Markowska-Daniel I, Pridotkas G, Nieto R, Fernandez-Pacheco P, Bokhan S, Nevolko O, Drozhzhe Z, Perez C, Soler A, Kolvasov D, Arias M (2014) Genetic variation among African swine fever genotype II viruses, eastern and central Europe. Emerg Infect Dis 20(9):1544–1547
8. Goller KV, Malogolovkin AS, Katorkin S, Kolbasov D, Titov I, Hoper D, Beer M, Keil GM, Portugal R, Blome S (2015) Tandem repeat insertion in African swine fever virus, Russia, 2012. Emerg Infect Dis 21(4):731–732
9. Sanna G, Dei Giudici S, Bacciu D, Angioi PP, Giammarioli M, De Mia GM, Oggiano A (2017) Improved strategy for molecular characterization of African swine fever viruses from Sardinia, based on analysis of p30, CD2V and I73R/I329L variable regions. Transbound Emerg Dis 64(4):1280–1286
10. Li L, Ren Z, Wang Q, Ge S, Liu Y, Liu C, Liu F, Hu Y, Li J, Bao J, Ren W, Zhang Y, Xu T, Sun C, Li L, Wang S, Fan X, Wu Z, Huang B, Guo H, Wu X, Wang Z (2019) Infection of African swine fever in wild boar, China, 2018. Transbound Emerg Dis 66(3):1395–1398