Genotypic Characteristics of African Swine Fever Virus Maintained in Various Outbreaks in Vietnam During 2019-2021

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Research Article

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Abstract

This study aimed to identify potential genetic diversity among African swine fever virus (ASFV) strains circulating in central and southern Vietnam. Thirty ASFV strains were collected from domestic pigs and convalescent pigs with ASFV-infected clinical signs from 19 different provinces of central and southern Vietnam during 2019–2021. A portion of the B646L (p72) gene and the entire E183L (p54), CP204L (p30), and B602L (CVR) genes were amplified, purified, and sequenced. Web-based BLAST and MEGA-X software were used for sequence analysis. Analysis of the partial B646L (p72) gene, the full-length E183L (p54) and CP204L (p30) genes, and the central hypervariable region (CVR) of the B602L gene sequence showed that all 30 ASFV isolates belonged to genotype II and were 100% identical to the previously identified strains in Vietnam and China. Analysis of the p72, p54, and p30 regions did not indicate any change in the nucleotide and amino acid sequences among these strains in 3 years of research. No novel variant was found in the CVR within the B602L gene. Analysis of the CVR showed that these ASFV strains belong to subgroup XXXII. The results of this study revealed that these ASFVs shared high homology with ASFV isolates detected previously in northern Vietnam and China. Taken together, the results of this study and a previous study in Vietnam showed high stability and no genetic diversity in the ASFV genome.

Introduction

African swine fever (ASF) is an infectious disease that is an extremely contagious viral hemorrhagic disease affecting domestic pigs and wild boars, with mortality rates reaching up to 100% [1]. ASFV, which is a notifiable disease as defined by the World Organization for Animal Health (OIE), has entailed severe repercussions for the entire global pig industry, as pigs are important sources of protein. Currently, neither therapy nor a vaccine is available against this disease, with the only biosecurity measure being essentially prevention, and rapid laboratory diagnosis and elimination of infected pig herds are required [2]. Since the first recognition of ASF in Kenya in the 1920s [3], the disease has been described in various countries around the world but remains endemic in Sardinia and eastern and southern Africa [4, 5]. Recently, countries in Europe and at least 11 countries in Asia have reported numerous ASF outbreaks [6]. In particular, in 2018, an outbreak of ASF was reported in China [7, 8] and then shortly after in Vietnam [9], which had a huge socioeconomic impact on the pork industry [10].

The causative agent is a DNA virus that is presently the sole member of the *Asfivirus* genus, belonging to the family *Asfarviridae* [11]. ASFV is a large icosahedral virus with a double-stranded DNA genome comprised of 170 to 193 kbp with 150 to 167 open reading frames depending on the strain [12]. The replication of ASFV has similarities with Poxvirus and Iridovirus, as all of them are DNA viruses that replicate in the cytoplasm [13]. The ASFV particle possesses a complex multilayered structure approximately 200 nm in diameter [14]. Twenty-four ASFV genotypes have been identified and designated I to XXIV [15, 16].

The key to understanding ASFV diversity, including its viral biology and evolution, is to dissect ASFV genetic and genomic variations [17]. Several specific genetic targets have been used to assess ASFV genetic diversity; however, three major and dominant gene fragments, the B646L gene-encoded capsid protein 72 (p72), the E183L gene-encoded envelope protein 54 (p54), and the CP204L gene-encoded phosphoprotein protein 30 (p30), are commonly used [18, 19]. Unfortunately, B646L (p72) genotyping analysis may not provide adequate typing resolution or the ability to discriminate between viruses of different biological phenotypes [20]. Intensifying genotypic resolution and genetic characterization has been achieved by the additional assessment of E183L (p54), CP204L (p30) and B602L (CVR) [19, 21–23]. The B602L gene (CVR) of ASFV is a hypervariable genetic marker that has been demonstrated to be useful for the high-resolution discrimination of ASFV. To achieve finer discrimination between the viruses, the variation and distribution of these amino acid repeats have been investigated using the primers CVR FL1/CVR FL2 [22].

In Vietnam, previous studies based on sequencing of the C-terminal region of the B64 6L (p72) gene have been reported [9, 24, 25], with samples collected mainly in North Vietnam at the beginning of the epidemic. Since the end of 2019, the disease has appeared in 63/63 provinces and cities and is a very widespread and complex epidemic in southern Vietnam. Therefore, a broader study of the genotypic characteristics of African swine fever virus in various outbreaks in Vietnam during 2019–2021 is essential to answer the question of the origin and diversity of the virus, in addition to providing the molecular epidemiology of the virus and a strategy for disease control.

Materials And Methods

Study design and samples

A total of 148 blood samples from sick pigs with typical clinical manifestations were collected from 30 pig farms located in 19 provinces where outbreaks were reported from 2019 to 2021 (Fig. 1). The sick pigs showed signs of high fever, collapse, cyanosis and skin hemorrhage and a high mortality. A total of 30/118 ASFV strains were randomly selected from farms (one per farm), and 1–3 strains from each province were used for sequencing and genotypic analysis. In addition, all previously published Vietnamese ASFV strains during this period were also included for the comprehensive analysis.

Extraction of genomic DNA

Total DNA was extracted from each blood sample by using the commercial Promega™ Wizard™ SV Genomic DNA Purification System (Promega, USA) according to the manufacturer's instructions.

Primers

Routine PCR was used to confirm ASF positivity, as recommended by the Office International des Epizooties (OIE, Paris, France) [26]. Then, primer pairs from previous studies were used to amplify the variable 3'-end of the B646L gene (p72), the entire E183L gene (p54), the CP204L gene (p30) and the B602L gene (CVR) for sequencing, as shown in Table 1.

Table 1
Primers used to amplify for ASFV sequencing

Genes	Primers	Sequences (5'-3')	Size of products	Refs
B646L	P72U	GGCACAAGTTCGGACATGT	478bp	[15]
	P72D	GTACTGTAACGCAGCACAG		
E183L	PPA722	CGAAGTGCATGTAATAACGTC	676bp	[23]
	PPA89	TGTAATTTTCATTGCGCCACAAC		
CP204L	P30F	ATGAAAATGGAGGTCATCTTCAAAAC	521bp	[4]
	P30R	AAGTTTAATGACCATGAGTCTTACC		
B602L	CVR-FL1	TCGGCCTGAAGCTCATTAG	Various size	[22]
	CVR-FL2	CAGGAACTAATGATGTTCC		

PCR amplification of the viral DNA

The genes encoding the p72, p54, p30, and CVR proteins were amplified in a 25 µl reaction volume containing 12 µl MyTaq™ HS Mix (Bioline, USA), 5 µl DNA, 1 µl of each 10 µM primer, and 6 µl free-DNA water. A positive control (DNA) from Vietnamese AFSVs that were isolated and sequenced in another study (unpublished) was used.

The thermocycling conditions for PCR detection of ASFV included a 10-min initial denaturation step of 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 50°C, and 30 s at 72°C, with a 7-min elongation step at 72°C [26]. Afterward, the PCR products were mixed with GelRed nucleic acid stain (Merck, Germany) and electrophoresed in a 1% agarose gel (Invitrogen, Thermo Fisher Scientific, USA) using a 1 Kb Plus Ladder (Invitrogen, Thermo Fisher Scientific, USA) to indicate the sizes of the amplification products.

Genetic sequencing and analysis

PCR products were purified using the TopPURE® PCR/Gel DNA Purification Kit (ABT Ltd.co, Vietnam) based on the manufacturer's recommendations. After purification, the products were sent to a sequencing laboratory (Macrogen, Korea). The raw (ABI) sequences were viewed and edited by Chromas version 2.6.6 to remove any ambiguous sequences and poor reads. Other ASFV sequences were obtained from the GenBank database (NCBI) based on BLASTN. The nucleotide sequences of the B646L, E183L, CP204L, and B602L genes of the field ASFV strains were aligned to build a phylogenetic tree to compare with previously published Vietnamese ASFV strains and with other published global sequences available in GenBank (NCBI) (Table S1), including some vaccine candidate strains that are being studied and likely to be commercialized in the near future in Vietnam and some countries where ASFV is circulating.

Nucleotide/amino acid alignment and phylogenetic analysis were conducted using the corresponding CLUSTAL W and MEGA X software programs based on the neighbor-joining method. To determine the degree of statistical support for each node in the resulting trees, the data were replicated 1000 times using the bootstrap method. The nucleotide sequences of the B646L (p72), CP204L (p30), E183L (p54), and B602L (CVR) genes from this study were deposited in GenBank under the accession numbers MW828850-MW828879, MW828820-MW828849, MW828760-MW828789 and MW828790-MW828819, respectively (Table 2).

Table 2
The field strains of ASFV collected for study

Strains	Year	Pigs	Provinces	Accession numbers obtained from Genbank			
				B646L(p72)	E183L(p54)	CP204L(p30)	B602L(CVR)
D/ASFV/VN/NA/2019	2019	DP	Nghe An	MW828877	MW8282769	MW828847	MW828817
D/ASFV/VN/Hue/2019	2019	DP	Hue	MW828857	MW8282779	MW828827	MW828797
D/ASFV/VN/DL/2019	2019	DP	Dak Lak	MW828854	MW8282776	MW828824	MW828794
D/ASFV/VN/KH/2019	2019	DP	Khanh Hoa	MW828873	MW8282765	MW828843	MW828813
D/ASFV/VN/BP/2019	2019	DP	Binh Phuoc	MW828851	MW8282773	MW828821	MW828791
D/ASFV/VN/BT/2019	2019	DP	Binh Thuan	MW828852	MW8282774	MW828822	MW828792
D/ASFV/VN/HCM/2019	2019	DP	Ho Chi Minh	MW828870	MW8282762	MW828840	MW828810
D/ASFV/VN/DN-LT/2019	2019	DP	Dong Nai	MW828871	MW8282763	MW828841	MW828811
D/ASFV/VN/DN-VC/2019	2019	DP	Dong Nai	MW828879	MW8282771	MW828849	MW828819
D/ASFV/VN/BD-PG2/2019	2019	CP	Binh Duong	MW828855	MW8282774	MW828825	MW828795
D/ASFV/VN/BD-PG3/2019	2019	DP	Binh Duong	MW828859	MW8282781	MW828829	MW828799
D/ASFV/VN/BD-PG4/2019	2019	CP	Binh Duong	MW828864	MW8282786	MW828834	MW828804
D/ASFV/VN/LA-BL1/2019	2019	DP	Long An	MW828860	MW8282782	MW828830	MW828800
D/ASFV/VN/LA-BL2/2019	2019	DP	Long An	MW828861	MW8282783	MW828831	MW828801
D/ASFV/VN/LA-BL3/2019	2019	DP	Long An	MW828862	MW8282784	MW828832	MW828802
D/ASFV/VN/CT/2019	2019	DP	Can Tho	MW828869	MW8282761	MW828839	MW828809
D/ASFV/VN/VL/2019	2019	DP	Vinh Long	MW828872	MW8282764	MW828842	MW828812
D/ASFV/VN/BRVT-CD/2019	2019	DP	Vung Tau	MW828874	MW8282766	MW828844	MW828814
D/ASFV/VN/BRVT-XM/2019	2019	DP	Vung Tau	MW828876	MW8282768	MW828846	MW828816
D/ASFV/VN/QN/2020	2020	DP	Quang Nam	MW828867	MW8282789	MW828837	MW828807
D/ASFV/VN/PY/2020	2020	DP	Phu Yen	MW828850	MW8282772	MW828820	MW828790
D/ASFV/VN/LD-DL/2020	2020	DP	Lam Dong	MW828875	MW8282767	MW828845	MW828815
D/ASFV/VN/LD-BL/2020	2020	DP	Lam Dong	MW828858	MW8282780	MW828828	MW828798
D/ASFV/VN/DN-TN/2020	2020	DP	Dong Nai	MW828868	MW8282760	MW828838	MW828808
D/ASFV/VN/DN-LK/2020	2020	CP	Dong Nai	MW828878	MW8282770	MW828848	MW828818
D/ASFV/VN/DN-TB/2020	2020	CP	Dong Nai	MW828856	MW8282778	MW828826	MW828796
D/ASFV/VN/BD-PG1/2020	2020	DP	Binh Duong	MW828853	MW8282775	MW828823	MW828793
D/ASFV/VN/TG/2020	2020	DP	Tien Giang	MW828863	MW8282785	MW828833	MW828803
D/ASFV/VN/BTr-GT/2020	2020	DP	Ben Tre	MW828865	MW8282787	MW828835	MW828805
D/ASFV/VN/GL/2021	2021	DP	Gia Lai	MW828866	MW8282788	MW828836	MW828806
DP: Domestic pig; CP: Convalescent pig (Domestic pig)							

Results

Molecular characteristics

B646L (p72)

The ASFV strains in this study shared 100% similarity at the nucleotide (nu) and amino acid (aa) levels, and the similarity with previous Vietnamese strains was 100%. The field ASFV strains had a high similarity (100%) at the nu and aa levels with strains belonging to genotype II, such as Chinese strains (Pig/HLJ/2018 and Wuhan/2019) and European and Eurasian strains (Belgium/Etalle/wb/2018, ASFV/LT14/1490, Pol18/28298/o111, and Georgia_2007/1). However, the field ASFV strains had differences at the nu and aa levels with genotype I strains of 0.84–1.05% and 0–0.63%, respectively.

E183L (p54)

The ASFV strains in this study shared 100% similarity at the nu and aa levels with each other and had 100% similarity with Vietnamese strains from previous studies. The field ASFV strains had a high similarity (100%) at the nu and aa levels with strains of genotype II, such as Chinese strains (Pig/HLJ/2018 and Wuhan/2019) and European and Eurasian strains (Belgium/Etalle/wb/2018, ASFV/LT14/1490, Pol18/28298/o111, and Georgia_2007/1) but differences of 2.18–8.65% and 1.64–12.44% with strains of genotype I, respectively.

CP204L (p30)

ASFV strains in this study shared 100% similarity at the nu and aa levels with each other and had 100% similarity with Vietnamese strains from previous studies. The field ASFV strains had a high similarity (100%) at the nu and aa levels with strains of genotype II, such as Chinese strains (Pig/HLJ/2018 and Wuhan/2019) and European and Eurasian strains (Belgium/Etalle/wb/2018, ASFV/LT14/1490, Pol18/28298/o111, and Georgia_2007/1) but of differences 2.32–2.50% and 1.61–2.68% with strains of genotype I, respectively.

B602L (CVR)

ASFV strains in this study shared absolute homology at the nu and aa levels, and the similarity with previous Vietnamese strains was 100%. The field ASFV strains had a high similarity (100%) at the nu and aa levels with strains belonging to genotype II, such as Chinese strains (Pig/HLJ/2018 and Wuhan/2019) and European and Eurasian strains (Belgium/Etalle/wb/2018, ASFV/LT14/1490, Pol18/28298/o111, and Georgia_2007/1). However, the field ASFV strains shared differences at the nu and aa levels with the genotype I strains at 3.45–10.62% and 3.97–12.7%, respectively.

In addition, the number of aa tandem repeats in the B602L gene region is a highly variable genetic marker, which was used to differentiate closely related p72 genotypes. The B602L gene of 30 ASFV strains in this study was successfully amplified and sequenced, and the results showed nu sequences of 400 bp, which corresponded in size and aa sequence to other genotype II strains with 10 aa tetramers, except for ZAM/14/Chipata (LC174766) with 14 aa tetramers. Interestingly, the field ASFV strains from this study belonged to the CVR subgroup XXXII based on the length and sequence of the aa tetramer repeats (Table 3). However, the quantity of aa tetramers of the CVR sequence differed from low to high among the field strains and the reference strains belonging to various genotypes, as presented in Table 3. ASFV strains with 10 aa tetramer repeats have been described from previous outbreaks in Vietnam, China, Belgium, and Georgia, indicating the close relationship between these viruses. The genetic variation within genotype II is barely detectable, even though the viruses in this study were sampled over 3 years.

Table 3
The aa-tetramer sequence within the central variable region (CVR) of gene B602L within ASFV strains

Strains virus	Accession no.	Isolates	P72 Genotype	Amino acid (aa) sequence of CVR fragment		CVR subgrc
				aa-tetramer sequence	Quantity	
VN/NA/2019-ASF ¹	-	Viet Nam	II	BNDBNDBNAA ²	10	XXXII
Hanoi/2019	MT166692	Viet Nam	II	BNDBNDBNAA	10	XXXII
NgheAn/2019	MT180393	Viet Nam	II	BNDBNDBNAA	10	XXXII
Wuhan/2019	MN393476	China	II	BNDBNDBNAA	10	XXXII
Pig/HLJ/2018	MK333180	China	II	BNDBNDBNAA	10	XXXII
Pol18/28298/o111	MT847621	Poland	II	BNDBNDBNAA	10	XXXII
Georgia_2007/1	NC044959	Georgia	II	BNDBNDBNAA	10	XXXII
Belgium/Etalle/wb/2018	MK543947	Belgium	II	BNDBNDBNAA	10	XXXII
ASFV/LT14/1490	MK628478	Lithuania	II	BNDBNDBNAA	10	XXXII
ZAM/14/Chipata	LC174766	Zambia	II	BNABNDBTDBNAAG	14	NK
OURT_88/3	AM712240	Portugal	I	ABNAAAAAAAAAAAAACCBNABNAAAAACBNABNABNABTDBNAFA	46	NK
Benin97/1	AM712239	Benin	I	ABNAAAACBNAAAAACBNAAAAACBNAAAAACBNNAFA	36	XIX
MUZUKI/1979	AY261362	South Africa	I	BVWAFNBNAAAF	12	NK
Warthog	AY261366	Namibia	IV	BNABUBMABUBMAAA	15	NK
Ken05/Tk1	NC044945	Kenya	X	AABNAABBA	9	XXVa
Ken06Bus	KM111295	Kenya	IX	AAABNABBNABBAABNABNABA	22	XXIV
ZAM/13/Kalomo	LC174767	Zambia	XIV	BNWNBFV	7	NK
R8	MH025916	Uganda	IX	AAAABNABBNABBAABBNABNABA	24	NK
RSA_2/2008	MN336500	South Africa	XXII	BVWVVNABNAABAGFFABAG	20	NK
Liv13/33	MN913970	France	I	BNADBNAFTBTDBNAF	16	NK
RSA/W1/1999	MN641876	South Africa	IV	BNABUBNABUBBMAAA	17	NK

¹The ASFV strain was representative in this study as all field strains had identical nucleotide sequences; ²Analysis of CVR amino acid sequences reveals tetrameric repeats that have been assigned single letter codes corresponding to each type. Tetramic aminoacid repeats that have been previously characterized in ASFV isolates include repeat code A = CAST/CVST/CTST/CASI, B = CADT/CTDT, C = GAST/GANT, D = CASM, F = CANT, G = CTNT, J = GTDT, K = CTSP, L = YTNT, M = NEDT, N = NVDT/NVGT, O = NANI/NADI/NASI, H = RAST, S = SAST, T = NVNT, U = NIDT/NTDT, V = NAST/NADT/NANT/NAVt, W = SADT/SVDT and Y = NTDTI [19, 22, 36]. *NK: Not known

Phylogenetic trees

All ASFV strains in this study and the reference strains were used to build a phylogenetic tree based on the nu sequences of B646L (p72), E183L (p54), and CP204L (p30) (Fig. 2). All ASFV strains of this study were demonstrated to belong to genotype II, together with the previous strains reported from Vietnam (Hanoi/2019-MT166692 and NgheAn/2019-MT180393) and the first strains reported in China (Pig/HLJ/2018-MK333180 and Wuhan/2019-MN393476), and in particular had a molecular relationship with strains considered to be the source of recent ASF outbreaks globally (Belgium/Etalle/wb/2018-MK543947, ASFV/LT14/1490-MK628478, Pol18/28298/o111-MT847621, and Georgia_2007/1-NC044959). With absolute similarity in the gene segments of B646L (p72), E183L (p54), CP204L (p30) and B602L (CVR), the ASFV strains of this study were not separated in the phylogenetic tree and displayed the same origin.

Genetic homology of the field ASFV with vaccine strains

The ASFV strains of this study shared a high similarity at the nu and aa levels with vaccine candidate strains belonging to genotype II, such as Pig/HLJ/18 and Georgia_2007/1 but a lower similarity with those such as Benin 97/1 and OURT_88/3, which belong to genotype I (Table 4). In B602L (CVR), the Pig/HLJ/18 and Georgia_2007/1 strains were similar to the Vietnamese field ASFV strains with 10 aa tetramers, while the OURT_88/3 and Benin 97/1 strains had 46 and 36 aa tetramers, respectively.

Table 4
Nucleotide (nu) and amino acid (aa) similarity (%) among Vietnamese ASFV strains compared with candidate vaccine strains

Vaccine strains		B646L/p72		E183L/p54		CP204L/p30		B602L/CVR	
Genotypes	Isolates	nu	aa	nu	aa	nu	aa	nu	aa
II	Pig/HLJ/18	100	100	100	100	100	100	100	100
	Georgia_2007/1	100	100	100	100	100	100	100	100
I	Benin 97/1	99.16	100	97.46	97.28	97.68	97.86	93.89	91.26
	OURT_88/3	99.16	100	97.46	97.28	97.5%	97.32	96.02	95.23

Discussion

African swine fever remains the major constraint on pig production in Vietnam, with outbreaks occurring throughout the year. There is a need for continuous characterization of ASFV strains during disease outbreaks to further understand ASF outbreak patterns and to map viral genotypes to the geographical regions within which they circulate. Continuous genotyping is important for giving insight into the virus origin, the epidemiological scenario and the early detection of variants and/or novel strains. Furthermore, knowledge of the molecular epidemiology of ASFV strains is necessary to develop effective prevention and control strategies at both local and regional levels. Thus far, through this study, it can be seen that ASFV strains in Vietnam have the same origin and maintain stable genetic characteristics. In addition, this study provides a comparison of genetic homology among Vietnamese strains and global vaccine candidate strains, especially the Georgia_2007/1 isolate currently used for active research and development of an attenuated vaccine that lacks the I177L gene, ASF-G-ΔI177L [27]. This attenuated vaccine is expected to be commercialized in Vietnam; therefore, to the author's knowledge, this may be the first commercial vaccine globally for ASFV.

In this study, molecular diagnosis methods were used to confirm and characterize ASFV strains. The virus strains of this study were not only from pigs with characteristic clinical signs and from deaths reported from outbreaks in several farms and provinces but also from previous research in Vietnam, and thus the study strains were highly representative of strains in Vietnam from 2019 to 2021. Phylogenetic analysis based on the nu sequences of the C-terminus of the 3 dominant and major gene regions (B646L, E183L, CP204L) showed that all 30 field Vietnamese ASFV strains belonged to genotype II, with absolute homology. Although CP204L (p30) is considered as the gene fragment for differentiating among closely related viruses [28], the findings of this study showed that several ASFV strains obtained from many different epidemic areas in Vietnam showed a remarkable genetic stability and could not be distinguished from closely related strains (such as those from China and Georgia) [9].

The CVR is capable of providing further resolution than any of the genes encoding p72, p54 or p30 [29]. Fragment size analysis has identified B602L as the most variable genome region [19]. The variable region of B602L contains aa tetramers that vary in number and type. Sequence analysis of the B602L gene from the field ASFV strains identified 5 different aa tetramer sequences encoded in this genome region. One of these tetramer sequences was CTST, which is one of the less common tetramer sequences [19]. With 10 aa tetramer repeats in CVR (BNDBNDBNAA), the ASFV strains in this study were classified into subgroup XXXII, along with the ASFV isolates in circulation in Vietnam (2019), the Chinese ASFV strains from outbreaks in 2018–2019, and other strains in Europe. At the same time, a study in India that performed B602L variable region analysis also showed that the tetrameric aa repeats in CVR are similar to other post-2007-p72-genotype II viruses reported from other Asian and European countries [30]. Although analysis of the CVR has identified it as the most variable genome region, Estonia isolates, belonging to the same genotype II classification based on the p72 genes and showing no difference in size and aa tetramers in the CVR genes, had the aa change of Y instead of C compared with the reference strain (Georgia_2007/1) [31]. However, field strains in this study revealed high conservation and no variation over 3 years.

Therefore, the virus causing disease in pig production in the southern areas is highly derived from the viruses first reported in North Vietnam; however, we do not exclude the theory that the origin of the virus spreading from China followed an illegal route as a result of live pig transportation and pork products due to the sell-off of pigs when the disease was widespread in epidemic areas [10].

There are a few concerns, but the genetic analysis of the four independent regions of ASFV sequences in this study and previous reports in Vietnam showed homogeneity and maintenance of the Vietnamese ASFV population [9, 25]. In addition, one study reported a finding that conflicts with this study, and other previous studies have reported that the gene sequence encoding p72 from an ASFV strain (VN/Pig/NA/1393; MN711740) showed point mutations in its sequence [32]. In general, the mutation rate of DNA viruses is much lower than that of RNA viruses and ranges between 10^{-8} and 10^{-6} substitutions per nucleotide per cell infection [33]. Moreover, the evolutionary rate of double-stranded DNA is difficult to estimate [34]. Accurate proofreading of the DNA polymerase and the virus-encoded base excision DNA repair system results in a low mutation rate in ASFV DNA.

Our results in this study can be an important basis to show the stability and maintenance of the ASFV genotype in infections over a short period of time unless there is further continuous invasion by novel strains from elsewhere [35]. Interestingly, some of the field strains collected from the same outbreaks in the subacute to chronic phase in surviving pigs also showed no difference at the nu and aa levels (unreported results). Three years after the first time ASFV entered the Vietnamese pig herds, the epidemiological characteristics changed considerably, and the circulation of the virus was scattered everywhere. There were several outbreaks with mild clinical signs and lower mortality; therefore, it is very important to continue the surveillance of ASFV at the genetic level at wider and deeper scales in various groups of pig populations with different clinical manifestations. Until then, the results of this study provide valuable genotypic characteristics of the current ASFVs circulating in Vietnam during 2019–2021, which can be useful for ASF control programs in Vietnam and neighboring countries.

Conclusion

This study confirmed that the current ASFVs circulating in Vietnam from 2019 to 2021 belong to genotype II, have complete genetic homology, and are derived from the same origin. The sequencing and analysis of the B646L(p72), E183L(p54), CP204L(p30), and B602L(CVR) genes over the years 2019, 2020 and 2021 showed the stability of this virus.

Declarations

Ethics approval and consent to participate

Permission to collect the sample and conduct the lab analysis and study were conducted in compliance with the institutional rules for the care and use of laboratory animals and using protocol approved by Ministry of Agriculture and Rural Development (MARD) Vietnam (TCVN 8402:2010) and under the acceptance and approval by Department of Animal Health, Vietnam (DAH) and the Ministry of Education and Training, Vietnam (MOET): 2867-QĐ BGDDT, dated 01/10/2020.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Map showing location of ASF outbreaks in Vietnam where field AFSV strains were obtained and characterized.

Figure 2

The phylogenetic trees of ASFV based on nucleotide sequences of genes encoding p72 (A), p54 (B), p30 (C). The Neighbor-Joining approach was used for construction of phylogenetic trees in MEGA X software (<https://www.megasoftware.net/>). Numbers along branches indicate bootstrap values >50% (1000 replicates). The red circles indicated ASFV strain in this study. Scale bars indicate nucleotide substitutions per site.

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