



Genetic Analysis of African Swine Fever Virus Originating from Pork Products in Indonesia

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Abstract | African swine fever (ASF) is a viral pig disease that has spread internationally and has a severe impact on pork production in the agricultural sector, trade economy, and social welfare in affected regions. In this context, pork products can potentially be a source of the spread owing to the long persistence of the causal virus. Therefore, this study aims to analyze ASFV genetically from pork product samples in 2019-2023 based on the B646L gene and determine the kinship relationship with reference ASFV in Indonesia and other countries. The study used 38 pork products collected in 2020-2023, consisting of 28 archived samples of ASFV positive, which were imported or transported between domestic regions. The other 10 were imported and local pork products purchased at e-commerce stores to represent samples that were already circulating in the community. Subsequently, the isolates were sequenced and compared with other ASFV isolates to establish the virus genotype. Sanger sequencing was conducted using the B646L gene, which was the most frequently used genetic marker. The phylogenetic tree construction results showed that genotype II was present in the samples, which was similar to Indonesian isolates and other Asian isolates. Therefore, the import of pigs and pork products must be regulated to prevent the entry of other genotypes and wider domestic spread.

Keywords | African swine fever, B646L, Gene, Indonesia, p72, Pork product

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INTRODUCTION

African swine fever (ASF) is a viral pig disease that has spread internationally and severely affects trade economy, social welfare, and pork production in the agricultural

sector (Bergmann *et al.*, 2022). According to the release of the International Committee on Taxonomy of Viruses EC 52 in October 2020, ASF virus (ASFV) is classified into the class Pokkesviricetes, order Asfuvirales, family Asfaviridae, and genus Asfivirus (ICTV, 2020). The virus retains

a double-stranded DNA (dsDNA) genome that measures 170-194 kilobases in length, inverted repeat sequences at the ends, and a hairpin structure. In addition, its genome has more than 150 open reading frames that encode different enzymes, such as structural proteins and multiple enzymes involved in DNA replication, gene transcription, and protein modification (Li *et al.*, 2022).

ASF was first discovered in Africa in 1921 (Montgomery, 1921), and the first outbreak in Europe occurred in Portugal in 1957 and spread to several Western European countries. The disease occurred in Eastern Europe, Georgia in 2007 and spread rapidly to neighboring countries (Cwynar *et al.*, 2019). In addition, China reported the first outbreak in 2018, which was transmitted to many Asian countries (WOAH, 2021). In mid-2021, ASF began to approach the Americas, with reports in the Dominican Republic and Haiti (CaribVET, 2021).

In line with previous reports, ASF occurred in Indonesia in early September 2019, with deaths reported in backyard pig in North Sumatra Province (Dharmayanti *et al.*, 2021). At the end of 2019, the government officially declared the entry of the disease into Indonesian territory (MoA, 2019), leading to the spread to 23 provinces in Indonesia (MoA, 2023). In this context, Dharmayanti *et al.* (2021) conducted nucleotide analysis of ASFV isolates from North Sumatra and West Java. The results showed that the 2 isolates were identical, belonging to genotype II based on a partial B646L gene. B646L gene encodes the main capsid protein p72 that contributes to viral structure. López-Otín *et al.* (1990) sequenced and analyzed the full length of the p72 encoding gene, laying the foundation for its exploration as a diagnostic gene. At present, 24 genotypes of ASFV have been detected, with the most recent description originating from Ethiopia (Achenbach *et al.*, 2017). All molecular studies on ASFV in Indonesia are from clinical samples, such as blood, swabs, and organs and none has been published regarding the genetic variation analysis from pork products.

Primatika *et al.* (2021) stated that a major obstacle faced by the government in controlling and preventing ASF outbreaks was monitoring pork products. These products can potentially be a source of ASF spread between countries and domestic regions owing to the long persistence of the virus. Fischer *et al.* (2020) stated that ASFV in frozen pork could last around 3 to 4 months, while food products could last 8 to 200 days, depending on the product type and storage. Several investigations have detected ASFV in confiscated pork products in Taiwan (Wang *et al.*, 2019), South Korea (Kim *et al.*, 2019) and Thailand (Songkasupa *et al.*, 2020). ASFV can survive in pig carcasses and meat products, such as Italian salami, pork belly, loin (Petrini *et al.*, 2019), bone marrow (Arzumanyan *et al.*, 2021), and meat

exudates (Onyilagha *et al.*, 2021). Therefore, this study aims to analyze ASFV genetically from pork product samples in 2019-2023 based on the B646L gene and see the kinship relationship with references in Indonesia and other countries. The findings could be used in further studies and policy input in the field of trade and control of pork products traffic due to their high potential as carriers of ASFV to countries or regions.

MATERIALS AND METHODS

SAMPLE COLLECTION

This study used 38 samples of pork products, which consisted of 25 archived ASFV positives belonging to the Center for Diagnostic Standard of Agricultural Quarantine (CDSAQ), and 3 archived ASFV positives from the Tanjung Priok Agricultural Quarantine Agency collected in 2020-2023. The 28 samples were pork products imported or transported between domestic regions and first tested in the animal quarantine laboratory. Meanwhile, the other 10 were purchased randomly at e-commerce stores to represent products circulating in the community. Permission to use archive samples for study purposes was approved through letter Number: 2388/KP.320/K.5.A/11/2023.

DNA EXTRACTION AND AMPLIFICATION

DNA was isolated from 100 mg of pork products using the High Pure Template Preparation Kit (Roche) following the protocol for isolating nucleic acids from mammalian tissues according to the manufacturer's instructions (Agüero *et al.*, 2003; Forth *et al.*, 2020). The primers used in this study partially amplified B646L (VP72) gene with the forward sequence 5'-CTG-CTC-ATG-GTA-TCA-ATC-TTA-TCG-A-3', the reverse sequence 5'-GAT-ACC-ACA-AGA-TC(AG)-GCC-GT-3' with an amplicon length of 250 bp (King *et al.*, 2003). In addition, the primers were widely recommended by WOA (2021).

All samples were tested using conventional PCR to obtain PCR products for genetic analysis. The Mastermix used KAPA2G Fast Readymix (Sigma Aldrich) with a composition of 2× KAPA2G Fast readymix 12.5 µL, forward and reverse primers each 1 µL (0.4 pmol), DNA template 5 µL, and nuclease-free water to ensure the total reaction volume reached 25 µL. Initial denaturation was carried out at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 10s, annealing at 60°C for 15s, extension at 72°C for 15s, and a final extension at 72°C for 60s. Samples that showed appropriate DNA bands were used for B646L gene sequencing. Subsequently, PCR products were sent to PT. Genetika Science as a provider of nucleotide sequencing services using Sanger method.

DATA ANALYSIS

Samples nucleotide sequence data were analyzed with information available on National Center for Biotechnology

Information (NCBI) website with isolates from Indonesia and other countries. Nucleotide sequence alignment and phylogenetic analysis were performed using MEGA 11 software (Tamura *et al.*, 2021). A phylogenetic tree was then constructed to determine the genotype and the relationship between the samples and references from Indonesia. These trees were constructed using the maximum likelihood method and the Kimura-2 parameter model with 1000 bootstrap replications, which was used to determine ASFV genotype previously (Anggy *et al.*, 2023; Duc Hien *et al.*, 2023; Qu *et al.*, 2022). Homology analysis compared the percent identity of Basic Local Alignment Search Tool (BLAST) results for the studied samples with ASFV sequence database from NCBI website.

RESULTS AND DISCUSSION

The 38 pork products used in this study consisted of 22 frozen and 16 processed meat samples. In addition, 28 showed positive PCR results, as indicated by a 250 bp band on agarose. When viewed per sample type, among 22 frozen meat, 20 positive test results were obtained, and from 16 processed meat, 8 positive test results were found (Figure 1). These results indicated that ASFV resistance in frozen meat was higher compared to processed meat. Previous studies stated that frozen meat ASFV could survive for 90–1000 days (Adkin *et al.*, 2004; Fischer *et al.*, 2020). The absence of processes, such as heating, salting, and curing, as well as storage at low temperatures, made the viability of the virus more stable. ASFV had been reported to have a high level of resistance to environmental conditions and could remain infective for a long period at temperatures below 0°C (Plowright and Parker, 1967). A total of 5 specimens were selected out of the 28 positive samples, for nucleotide sequencing, and this was based on the year of collection, sample type, and visible DNA bands. Information on these samples is presented in Table 1.

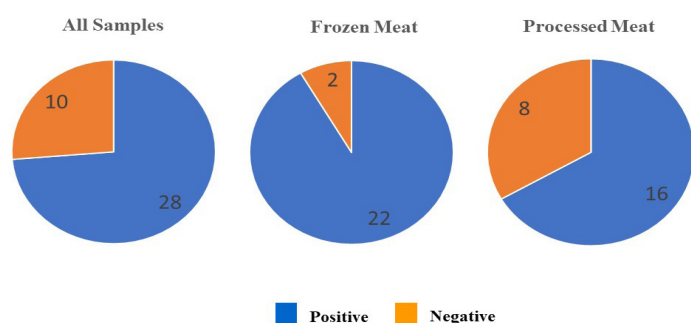


Figure 1: Pie chart showing the results of the PCR test for ASFV detection for the total samples in this study (n=38) and by sample type.

The genetic relationship of the samples with ASFV isolates from other genotypes was analyzed by constructing a phylogenetic tree based on B646L gene. The results of

phylogenetic tree construction of the samples and 24 other genotypes are shown in Figure 2. The results showed that all samples belonged to genotype II. This study reinforced several reports, stating that ASFV circulating in Indonesia was genotype II, and no other genotypes were found, either based on B464L (p72) gene or E183L (p54) gene (Anggy *et al.*, 2023; Sima and Kharisma, 2023; Dharmayanti *et al.*, 2021; Sanam *et al.*, 2022).

Table 1: Sample information.

Sample code	Sample identity	Type	Collect-ed year
A	ASFV/Meat/local/2020	Frozen Meat	2020
B	ASFV/Driedmeat/local/2020	Dried Meat	2021
D	ASFV/Bacon/ecom/2022	Bacon	2022
E	ASFV/Meat/import/2023	Frozen Meat	2023
F	ASFV/Meat/local/2023	Frozen Meat	2023

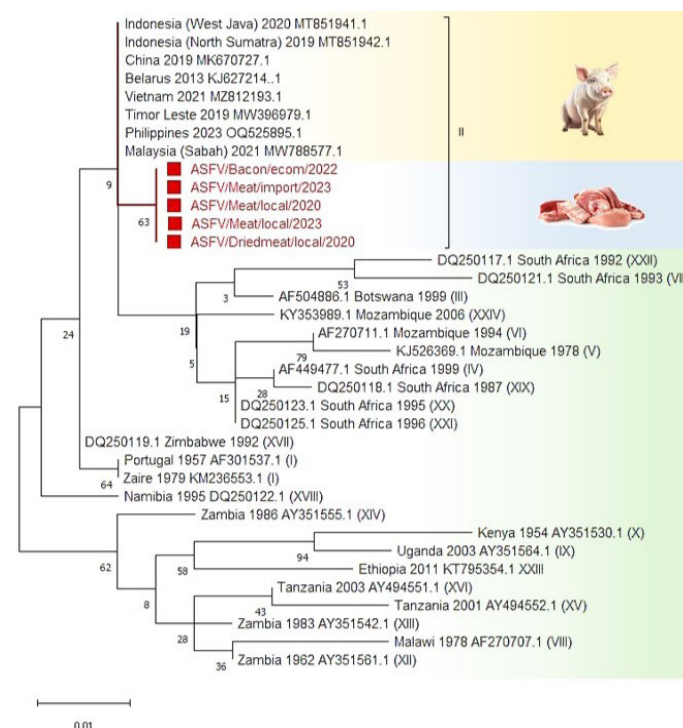


Figure 2: The phylogenetic tree of the African swine fever virus is based on the B646L gene (p72) with 250 bp in size. Phylogenetic analysis was performed using the maximum likelihood method, bootstrap value 1000, and Kimura-2 parameter model. The ■ sign and red text indicates samples from this study. GenBank accession numbers and genotype are provided for reference sequences.

Figure 3 revealed the relationship between pork product samples and 4 reference Indonesian isolates from 2019 to 2023, and this came from swab samples, blood, and organs of sick pig. Despite their inclusion in genotype II, these samples were reported to be different clusters. The existence of branch differences between the pork products and the reference isolates from Indonesia was due to differences

Table 2: Sequence similarity of the studied samples with sequences from the NCBI database.

Accession number	Country of origin	Year of collection	Sequence similarity (%)				
			A*	B	C	D	E
OP781309.1	Madagascar	1998	99,60	99,58	99,20	99,58	99,20
OP781313.1	Zimbabwe	2005	99,60	99,58	99,20	99,58	99,20
OR428151.1	Indonesia	2019	99,60	99,58	99,20	99,58	99,20
OR660699.1	Serbia	2022	99,60	99,58	99,20	99,58	99,20
OR604566.1	Indonesia	2023	99,60	99,58	99,20	99,58	99,20

The * sign indicates the sample code. The identities of the samples are listed in Table 1.

in several nucleotides. The discrepancies could be caused by the effects of mutagens, in this case, due to physical and chemical processes, which reacted with DNA and change the structure of individual nucleotides (Brown, 2002).

Phylogenetics was an excellent approach to understanding the evolution of species. By studying phylogenetic trees, investigators better understood how species evolved while explaining the similarities and differences between types. Phylogenetic studies could help analyze the evolution and similarities between diseases and viruses as well as create vaccines to prevent these diseases (Mahajani *et al.*, 2019).

Homology analysis of the results of sequencing report samples with ASFV sequence database from NCBI was performed to determine sequence similarity. A total of 5 sequence datasets were obtained from NCBI database collections for 1998, 2005, 2019, 2022, and 2023. All reference isolates showed stable sequence similarity, ranging from 99.20%-99.60% to the samples studied (Table 2). This study revealed that ASFV from pork products in Indonesia had only experienced very few nucleotide changes compared to reference isolates circulating from 1998 to 2023. This was in line with the statement that ASF virus was indeed a virus with a low genome mutation rate, a high level of genetic stability, and evolved very slowly (Cho *et al.*, 2023; Forth *et al.*, 2023; Qu *et al.*, 2022).

Spontaneous mutation rates between viruses vary widely, and some viruses had the extraordinary ability to adapt to new hosts and environments. RNA viruses mutate more quickly than DNA viruses because RNA viruses were single-stranded, and DNA viruses were double-stranded. Furthermore, genome size was also negatively correlated with mutation rate (Sanjuán and Domingo-Calap, 2016). The large size of ASFV genome was the main factor in the slow virus mutation rate. Although the overall genome mutation rate of ASFV was relatively low, ASFV exhibited genetic and antigenic diversity. However, to date, no study had been able to distinguish the origin of ASFV transmission with certainty by analyzing only the genotype. More complete genome analysis and epidemiological studies were needed to estimate the origin of the virus.

Analysis of ASFV evolution was mostly carried out based on the conserved B646L gene, and this was gradually becoming the most important typing criterion (Qu *et al.*, 2022). Investigating the molecular characteristics of ASFV during outbreaks was crucial for differentiating between closely related strains, understanding the virus's origin, and expanding knowledge of the molecular evolution and epidemiology (Giammarioli *et al.*, 2023; Xiong *et al.*, 2021).

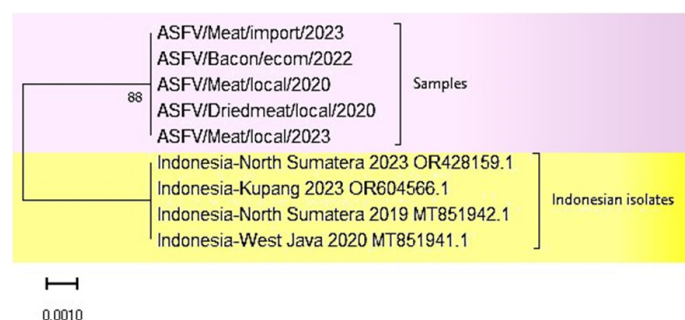


Figure 3: Phylogenetic tree of studied samples and reference isolates from Indonesia collected in 2019-2023 based on the B646L gene (p72). Phylogenetic analysis was performed using the maximum likelihood method, bootstrap value 1000, and Kimura-2 parameter model.

The first appearance of Genotype II was recorded in Georgia in 2007, and it subsequently had a significant impact on the swine industry and the global economy due to ASF pandemic (Mazloun *et al.*, 2023). Clinical manifestation, morbidity, and mortality in domestic pig and wild boar caused by ASFV genotypes I and II were similar, as demonstrated in numerous animal experiments (Blome *et al.*, 2013). ASF in Asia was first reported in China in 2018 and had spread to other Asian countries, including Southeast Asia, such as Indonesia, Vietnam, Timor Leste, Laos, Myanmar, Cambodia, and the Philippines in 2019, Malaysia in 2021, and Singapore in 2023 (FAO, 2023). According to Sur's (2019) historical analysis, pork illegal import and export were identified as the primary cause of the widespread ASF outbreaks in multiple countries. Fischer *et al.* (2020) summarized several in-vivo and in-vitro experimental studies on the resistance of ASF virus in pork products. Since ASFV was highly stable in pork products, it could be transmitted whenever such contaminated products were used as swill (Nuanalsuwan *et al.*, 2022).

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NOVELTY STATEMENT

No research has been conducted regarding the genetic variation of ASFV from pork products in Indonesia. The findings could be used in further studies and policy input in the field of trade and control of pork products traffic due to their high potential as carriers of ASFV to countries or regions.

AUTHOR'S CONTRIBUTIONS

SS, SA, IWT, NLP, HN: Equal authors, conducted the study, conceptualized the study, data analysis, and finalized the manuscript. The authors have read, reviewed, and approved the final content of the manuscript and agree to the conditions outlined in the copyright assignment form.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

REFERENCES

- CaribVET, Caribbean Animal Health Network (2021). African swine fever is present in the Caribbean! <https://www.caribvet.net/news/african-swine-fever-is-present-in-the-caribbean>
- FAO, Food and Agricultural Organization (2023). ASF Situation Update in Asia Pacific. <https://www.fao.org/animal-health/situation-updates/asf-in-asia-pacific/en>
- ICTV, International Committee on taxonomy of Virus (2020). African Swine Fever Virus. https://talk.ictvonline.org/taxonomy/p/taxonomy-history?src=NCBI&ictv_id=19810440
- MoA, Ministry of Agriculture (2019). Kepmentan No 820/Kpts/HC.32b/M/T2/2019 tentang Pernyataan Wabah Penyakit Demam Babi Afrika (African Swine Fever) Pada Beberapa Kabupaten/Kota di Propinsi Sumatera Utara.2019.
- MoA, Ministry of Agriculture (2023). Kepmentan No 311/KPTS/PK.320 /M/06 /2023 tentang Penetapan Status Situasi Penyakit Hewan.
- WOAH, World Organization For Animal Health (2021). Chapter 3.9.1: African Swine Fever (Infection With African Swine Fever Virus. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.09.01_ASF.pdf
- Achenbach JE, Gallardo C, Nieto-Pelegrín E, Rivera-Arroyo B, Degefa-Negi T, Arias M, Jenberie S, Mulisa DD, Gizaw D, Gelaye E, Chibssa TR, Belaye A, Loitsch A, Forsa M, Yami

Adkin *et al.* (2004), in a risk assessment regarding illegal imports of meat and meat products, stated that residual pork products through aircraft or ship waste were involved in previous ASFV outbreaks outside Africa in 1960-1985, however, this case has not been proven. According to Goggin *et al.* (2013), it was concluded that the uncontrolled movement of pork products contaminated with ASF and swill feeding caused the spread of ASF in the Russian Federation during 2007-2012. Sánchez-Vizcaíno *et al.* (2015) also stated that transporting infected products was the most frequent route for spreading ASF. Wang *et al.* (2018) posited that the presence of ASF in China could potentially be attributed to the smuggling of pork or offal from ASF-infected Eastern European countries. Sendow *et al.* (2020) stated that swill feed containing pork products contaminated with ASF could spread and increase the risk of it entering a country, including Indonesia. Pig farmers widely used swill feeding because it was relatively inexpensive compared to commercial feed. Swill feeding posed a very high risk of introducing several diseases into healthy populations, and there was an effective ban on it. Subsequently, pig must not be fed swill containing pork and must be boiled for 30 min with periodic stirring and cooled before feeding (Beltrán-Alcrudo *et al.*, 2017).

This study proved that ASFV genotype from pork products was the same as the virus circulating in Indonesia in 2019-2023. Pork products were considered the source of ASFV entering Indonesia, given the absence of live pig imports. This could also be carried in passenger carry-on bags on planes or ships, and food waste containing pork products. Furthermore, the illegal entry of live animals could also be a problem, considering that Indonesia was an archipelagic country with many borders. Although Indonesia was no longer free from ASF, regulations for monitoring and inspecting the import of pig and pork products must continue to be implemented to prevent the entry of other genotypes and wider domestic spread.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, B646L gene analysis showed that ASFV isolates from pork products were genotype II, similar to the Indonesian isolates in previous studies and other Asian isolates. Although Indonesia was no longer free from ASF, regulations for monitoring and inspecting the import of pig and pork products must continue to be implemented to prevent the entry of other genotypes and wider domestic spread. Despite the absence of studies on ASFV in pork products in Indonesia, the genetic analysis using other genetic markers was needed for a more complete characterization. In this context, epidemiological studies and viral viability with culture were needed to strengthen the potential of pork products as carriers of AFSV.

- M, Diallo A, Soler A, Lamien CE, Sánchez-Vizcaíno JM (2017). Identification of a New Genotype of African Swine Fever Virus in Domestic Pigs from Ethiopia. *Transboundary Emerg. Dis.*, 64(5): 1393–1404. <https://doi.org/10.1111/tbed.12511>
- Adkin A, Coburn H, England T, Hall S, Hartnett E, Marooney C, Wooldridge M, Watson E, Cooper J, Cox T, Seaman M (2004). Risk assessment for the illegal import of contaminated meat and meat products into Great Britain and the subsequent exposure of GB livestock (IIRA): foot and mouth disease (FMD. In New Haw: Vet. Lab. Agency, Agüero M, Fernández J, Romero L, Mascaraque CS, Arias M, Sánchez-Vizcaíno JM (2003). Highly Sensitive PCR Assay for Routine Diagnosis of African Swine Fever Virus in Clinical Samples. *J. Clin. Microbiol.*, 41(9): 4431–4434. <https://doi.org/10.1128/JCM.41.9.4431-4434.2003>
- Anggy FP, Nugroho WS, Irianingsih SH, Enny S, Srihanto EA (2023). Genetic analysis of African swine fever viruses based on E183L (p54) gene circulating in South Sumatra and Lampung province Indonesia. *Vet. World* 16(9): 1985–1990. <https://doi.org/10.14202/vetworld.2023.1985-1990>
- Arzumanyan H, Hakobyan S, Avagyan H, Izmailyan R, Nersisyan N, Karalyan Z (2021). Possibility of long-term survival of African swine fever virus in natural conditions. *Vet. World*, 14(4): 854–859. <https://doi.org/10.14202/vetworld.2021.854-859>
- Beltrán-Alcrudo D, Arias M, Gallardo C, Kramer S, Penrith ML (2017). African swine fever: detection and diagnosis-a manual for veterinarians. In Food Agric. Organ. United Nations Rome,
- Bergmann H, Dups-Bergmann J, Schulz K, Probst C, Zani L, Fischer M, Gethmann J, Denzin N, Blome S, Conraths FJ, Sauter-Louis C (2022). Identification of Risk Factors for African Swine Fever: A Systematic Review. *Viruses*, 14(10): 1–21. <https://doi.org/10.3390/v14102107>
- Blome S, Gabriel C, Beer M (2013). Pathogenesis of African swine fever in domestic pigs and European wild boar. *Virus Res.*, 173(1): 122–130. <https://doi.org/10.1016/j.virusres.2012.10.026>
- Brown T (2002). Mutation Repair and Recombination. In *Genomes* (2nd ed.). Oxford: Wiley-Liss.
- Cho KH, Yoo DS, Hong SK, Kim DY, Jang MK, Kang HE, Kim YH (2023). Genetic Profile of African Swine Fever Viruses Circulating at Pig Farms in South Korea during the Outbreaks between 2022 and April 2023. *Viruses*, 15(7): 1552. <https://doi.org/10.3390/v15071552>
- Cwynar P, Stojkov J, Wlazlak K (2019). African swine fever status in Europe. *Viruses*, 11(4): 1–17. <https://doi.org/10.3390/v11040310>
- Dharmayanti NLPI, Sendow I, Ratnawati A, Settypalli TBK, Saepulloh M, Dundon WG, Nuradji H, Naletoski I, Cattoli G, Lamien CE (2021). African swine fever in North Sumatra and West Java provinces in 2019 and 2020 Indonesia. *Transboundary Emerg. Dis.*, 68(5): 2890–2896. <https://doi.org/10.1111/tbed.14070>
- Duc Hien N, Trung Hoang L, My Quyen T, Phuc Khanh N, Thanh Nguyen L (2023). Molecular Characterization of African Swine Fever Viruses Circulating in Can Tho City Vietnam. *Vet. Med. Int.*, <https://doi.org/10.1155/2023/8992302>
- Fischer M, Hühr J, Blome S, Conraths FJ, Probst C (2020). Stability of African swine fever virus in carcasses of domestic pigs and wild boar experimentally infected with the ASFV isolate. *Viruses*, 12(10): 1118. <https://doi.org/10.3390/v12101118>
- Forth JH, Calvelage S, Fischer M, Hellert J, Sehl-ewert J, Roszyk H (2023). Afr. swine fever virus – variants on the rise. 12. <https://doi.org/10.1080/22221751.2022.2146537>
- Forth JH, Forth LF, Václavěk P, Barták P, Höper D, Beer M, Blome S (2020). Whole-Genome Sequence of an African Swine Fever Virus Isolate from the Czech Republic. *Microbiol. Res. Announcements*, 9(44): 11–12. <https://doi.org/10.1128/MRA.00948-20>
- Giammarioli M, Alessandro D, Cammà C, Masoero L, Torresi C, Maracchi M, Zoppi S, Curini V, Rinaldi A, Rossi E, Casciari C, Pela M, Pellegrini C, Iscaro C, Feliziani F (2023). Molecular Characterization of the First African Swine Fever Virus Genotype II Strains Identified from Mainland Italy 2022. *Pathogens*, 12(3): 372. <https://doi.org/10.3390/pathogens12030372>
- Gogin A, Gerasimov V, Malogolovkin A, Kolbasov D (2013). African swine fever in the North Caucasus region and the Russian Federation in years 2007–2012. *Virus Res.*, 173(1): 198–203. <https://doi.org/10.1016/j.virusres.2012.12.007>
- Kim HJ, Lee MJ, Lee SK, Kim D, young Seo SJ, Kang HE, Nam HM (2019). African swine fever virus in pork brought into South Korea by travelers from China August 2018. *Emerg. Infect. Dis.*, 25(6): 1231–1233. <https://doi.org/10.3201/eid2506.181684>
- King DP, Reid SM, Hutchings GH, Grierson SS, Wilkinson PJ, Dixon LK, Bastos ADS, Drew TW (2003). Development of a TaqMan® PCR assay with internal amplification control for the detection of African swine fever virus. *J. Virol. Methods*, 107(1): 53–61. [https://doi.org/10.1016/S0166-0934\(02\)00189-1](https://doi.org/10.1016/S0166-0934(02)00189-1)
- Li Z, Chen W, Qiu Z, Li Y, Fan J, Wu K, Li X, Zhao M, Ding H, Fan S, Chen J (2022). Afr. Swine Fever Virus : A Review. 1–41. <https://doi.org/10.3390/life12081255>
- López-Otín C Freije JMP, Parra F, Mendez E, Viñuela E (1990). Mapping and sequence of the gene coding for protein p72 the major capsid protein of African swine fever virus. *Virology*, 175(2): 477–484. [https://doi.org/10.1016/0042-6822\(90\)90432-Q](https://doi.org/10.1016/0042-6822(90)90432-Q)
- Mahajani A, Pandya V, Maria I, Sharma D (2019). *Phylogenetics Algorithms and Applications* (Vol. 904 Issue July). Springer Singapore.
- Mazloun A, van Schalkwyk A, Chernyshev R, Igolkin A, Heath L, Sprygin A (2023). A Guide to Molecular Characterization of Genotype II African Swine Fever Virus: Essential and Alternative Genome Markers. *Microorganisms*, 11(3): 1–15. <https://doi.org/10.3390/microorganisms11030642>
- Montgomery RE (1921). On A Form of Swine Fever Occurring in British East Africa (Kenya Colony). *J. Comp. Pathol.*, 34: 159–191. [https://doi.org/10.1016/S0368-1742\(21\)80031-4](https://doi.org/10.1016/S0368-1742(21)80031-4)
- Nuanualsuwan S, Songkasupa T, Boonpornprasert P, Suwankitwat N, Lohlamoh W, Nuengjamnong C (2022). Thermal Inactivation of African Swine Fever Virus in Swill. *Front. Vet. Sci.*, 9: 1–8. <https://doi.org/10.1186/s40813-022-00277-8>
- Onyilagha C, Nash M, Perez O, Goolia M, Clavijo A, Richt JA,

- Ambagala A (2021). Meat exudate for detection of african swine fever virus genomic material and anti-asfv antibodies. *Viruses*, 13(9). 1774. <https://doi.org/10.3390/v13091744>
- Petrini S, Feliziani F, Casciari C, Giammarioli M, Torresi C, De Mia GM.(2019). Survival of African swine fever virus (ASFV) in various traditional Italian dry-cured meat products. *Prev. Vet. Med.*, 162: 126–130. <https://doi.org/10.1016/j.prevetmed.2018.11.013>
- Plowright W, Parker J (1967). The stability of African swine fever virus with particular reference to heat and pH inactivation. *Arch. Gesamte Virusforsch.*, 21(3–4): 383–402. <https://doi.org/10.1007/BF01241738>
- Primatika RA, Sudarnika E, Sumiarto B, Basri C, Studi Kesehatan Masyarakat Veteriner P, Kedokteran Hewan F, Pertanian Bogor I, Barat J, Kesehatan Masyarakat Veteriner D, Gadjah Mada U (2021). Tantangan dan Kendala Pengendalian African Swine Fever (ASF) Challenges and Barriers to African Swine Fever (ASF) Control. 39(1). <https://doi.org/10.22146/jsv.61084>
- Qu H, Ge S, Zhang Y, Wu X, Wang Z (2022). A systematic review of genotypes and serogroups of African swine fever virus. *Virus Genes*, 58(2): 77–87. <https://doi.org/10.1007/s11262-021-01879-0>
- Sanam MU, Gelolodo M, Toha L (2022). Analisis Nukleotida dan Homologi Sekuens Fragmen Gen p72 (B646L) Virus African Swine Fever Virus (ASF) Asal Kota Kupang. *J. Kajian Vet.*, 10(2): 165–175. <https://doi.org/10.35508/jkv.v10i2.7875>
- Sánchez-Vizcaíno JM, Mur L, Gomez-Villamandos JC, Carrasco L (2015). An update on the epidemiology and pathology of African swine fever. *J. Comp. Pathol.*, 152(1): 9–21. <https://doi.org/10.1016/j.jcpa.2014.09.003>
- Sanjuán R Domingo-Calap P (2016). Mechanisms of viral mutation. *Cell. Mol. Life Sci.*, 73(23): 4433–4448. <https://doi.org/10.1007/s00018-016-2299-6>
- Sendow I, Ratnawati A, Dharmayanti NI, Saepullo M (2020). African Swine Fever: Penyakit Emerging yang Mengancam Peternakan Babi di Dunia. *Indones. Bull. Anim. Vet. Sci.*, 30(1): 15. <https://doi.org/10.14334/wartazoa.v30i1.2479>
- Sima PMA, Kharisma VD (2023). Identifikasi Persebaran Genotip Africa swine virus fever (ASFV) di Indonesia : Mini Review. *Prosiding Seminar Nasional Biologi FMIPA UNM Inovasi Sains Dan Pembelajarannya: Tantangan Dan Peluang* 118–124.
- Songkasupa T, Dokphut A, Boonpornprasert P (2020). Detection of African swine fever virus in confiscated pork products brought into Thailand during 2018 –2019. *The Thai Vet. Med.*, 257–259.
- Sur JH (2019). How far can African swine fever spread? *J. Vet. Sci.*, 20(4): 1–5. <https://doi.org/10.4142/jvs.2019.20.e41>
- Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.*, 38(7): 3022–3027. <https://doi.org/10.1093/molbev/msab120>
- Wang T, Sun Y, Qiu HJ (2018). African swine fever: An unprecedented disaster and challenge to China. *Infect. Dis. Poverty*, 7(1): 3–7. <https://doi.org/10.1186/s40249-018-0495-3>
- Wang WH, Lin CY, Chang Ishcol MR, Urbina AN, Assavalapsakul W, Thitithanyanont A, Lu PL, Chen YH, Wang SF (2019). Detection of African swine fever virus in pork products brought to Taiwan by travellers. *Emerg. Microbes Infection*, 8: 1000–1002. <https://doi.org/10.1080/22221751.2019.1636615>
- Xiong D, Zhang X, Xiong J, Yu J, Wei H (2021). Rapid genome-wide sequence typing of African swine fever virus based on alleles. *Virus Res.*, 297: 198357. <https://doi.org/10.1016/j.virusres.2021.198357>