

Genetic analysis of African swine fever virus in Bali based on p72 and CD2v Genes

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Abstract

African Swine Fever (ASF) has caused negative effects in the swine industry in various countries of the world, including Indonesia. Bali is one of the most severely affected provinces in Indonesia. Phylogenetic study to determine the genotype and serogroup of the ASF virus found in Bali has never been done before. In this study, virus was obtained from an infected spleen sample, designated as Bali/2023, and two gene fragments, namely p72 (B646L) and CD2v (EP402R), were then amplified by PCR and sequenced by Sanger method. The DNA sequences of p72 and CD2v virus was then compared with sequences from other countries for genetic analysis. The results showed that Bali/2023 virus is genetically very close to viruses found in other Asian countries, mainly viruses isolated in China and Vietnam in 2019. These genetic analyses results placed Bali/2023 virus in genotype II and serogroup 8.

Keywords: ASF; Virus; Bali/2023; p72; cd2v

1. Introduction

African Swine Fever (ASF) is a viral hemorrhagic disease affecting the species in the Suidae family (Zhou *et al.*, 2023) with extremely high mortality rate (Beltrán-Alcrudo *et al.*, 2017; Yang *et al.*, 2023b). Since it was first reported in Kenya in 1921 and the disease has now been found in South Africa, Eastern Europe, Latin America, East and South East Asia. (Ramirez-Medina *et al.*, 2022; Yang *et al.*, 2023a).

The ASF Virus (ASFV) belongs to the family Asfarviridae, genus *Asfivirus* (Indrabalan *et al.*, 2021). The virus has icosahedral structure with a diameter of roughly 200 nm (Tilocca *et al.*, 2023). In its nucleus, the genome is linear double-stranded DNA (dsDNA) encoding 150 to 200 different proteins (Li *et al.*, 2022). Two gene fragments that are often studied are p72 (B646L) and CD2v (EP402) that encode proteins found in the capsid and the outer membrane, respectively (Liu *et al.*, 2022; Miao *et al.*, 2023). Genotype and serogroup of the virus are also determined by analyses on these two gene fragments (Yin *et al.*, 2022).

In Indonesia, the first positive case of ASF was reported in 2019 in North Sumatera (Kolambani *et al.*, 2022). As of 2023, various other provinces have also been confirmed to be ASF positive, including Bali. Considering the effects of ASF in Bali's swine industry, research and studies on ASF are deemed highly important however phylogenetic study of the virus has never been done before. In this study, gene fragments p72 and CD2v were sequenced and genetically analyzed in order to determine the phylogeny of the ASFV found in Bali.

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2. Material and methods

The sample for this study was obtained from an infected pig spleen of an ASF positive case with registration number 533/N/23. Isolation of the viral DNA was done with GeneJet Genomic DNA Purification Kit, following the set procedures. The isolated DNA was then used for PCR with a master mix consisting of 5 μ l Taq polymerase mix (Bioline), 2 μ l of DNA and 10 nanomolar of primers. Primers for p72 gene fragment were F72 (ATGGCATCAGGAGGAGCTTTTGTCTT) and R72 (GGTACTGTAACGCAGCACAGCTGAACC), while primers used for CD2v gene fragment were FCD2v (ATGATAAATACTTATTTTTTAATATTTTCTAAC) and RCD2v (AATAATTCTATCTACGTGAATAAGCG). The PCR result was then brought for electrophoresis in 1% agarose gel in ethidium bromide and visualized under ultraviolet light. Sequencing of PCR products was conducted using Sanger Chain Termination method. Primary and secondary data (obtained from GenBank) were then aligned and analyzed using Mega7 application. In phylogenetic analysis, the method used was Maximum Likelihood Tree with Bootstrap 1000 times replication and Kimura 2 Parameter model (Kimura, 1980; Kumar *et al.*, 2016)

3. Results and discussion

Sequencing results showed good quality sequences with clean background. For gene fragment p72, a sequence of 1842 bp was obtained and for gene fragment CD2v, 967 bp. These data were deposited to GenBank and assigned accession number of PP078879 (p72) and PP078878 (CD2v).

The results of p72 genetic analysis showed that closest genetic distance between Bali/2023 virus and viruses from other countries is 0.001, while the furthest genetic distance is 0.017. Analysis result on nucleotide polymorphism showed a total of 71 polymorphic sites. The ASF virus found in Bali is genetically very similar to viruses found in other countries in Asia. While on amino acid level there are a total of seven polymorphic sites, with Bali/2023 virus having only one polymorphic site when compared to China/2019 virus.

Phylogenetic analysis result is shown on Figure 1. It can clearly be seen that Bali/2023 is in the same cluster with other viruses known to be genotype II, such as Vietnam/2022 and China/2019.

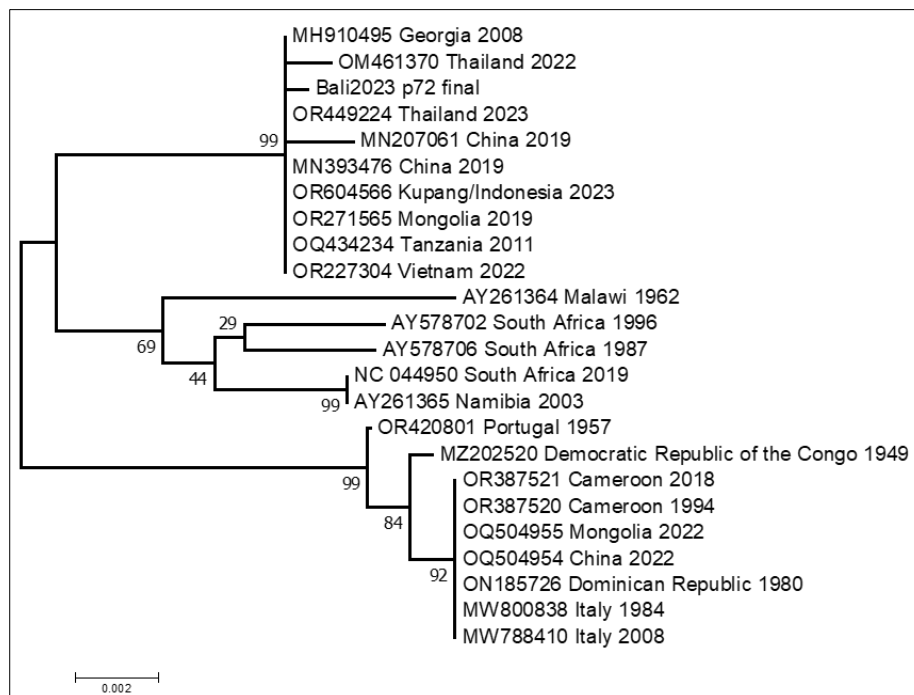


Figure 1 Molecular phylogenetic analysis result of p72 gene fragment using Maximum Likelihood Tree method based on Kimura 2 model parameter. The accession number, country of origin and year of isolation are shown in the taxon names

Genetic analysis results of gene fragment CD2v gave the closest genetic distance of 0.000 while the furthest is 0.005. It can be seen that there are no polymorphic sites between Bali/2023 virus and viruses found in many other Asian countries. Analysis with viruses found in Europe and Africa showed a total of 19 polymorphic sites. On amino acid polymorphism analysis there are a total of 14 polymorphic sites. The Bali/2023 virus has homologous amino acid sequence with other viruses from Asia. Phylogenetic analysis did not give conclusive result, as clusters did not form, as seen on Figure 2. However, this phylogenetic analysis placed Bali/2023 virus is very close to Vietnam/2019 virus that is known to belong in serogroup 8 (Le et al., 2019)

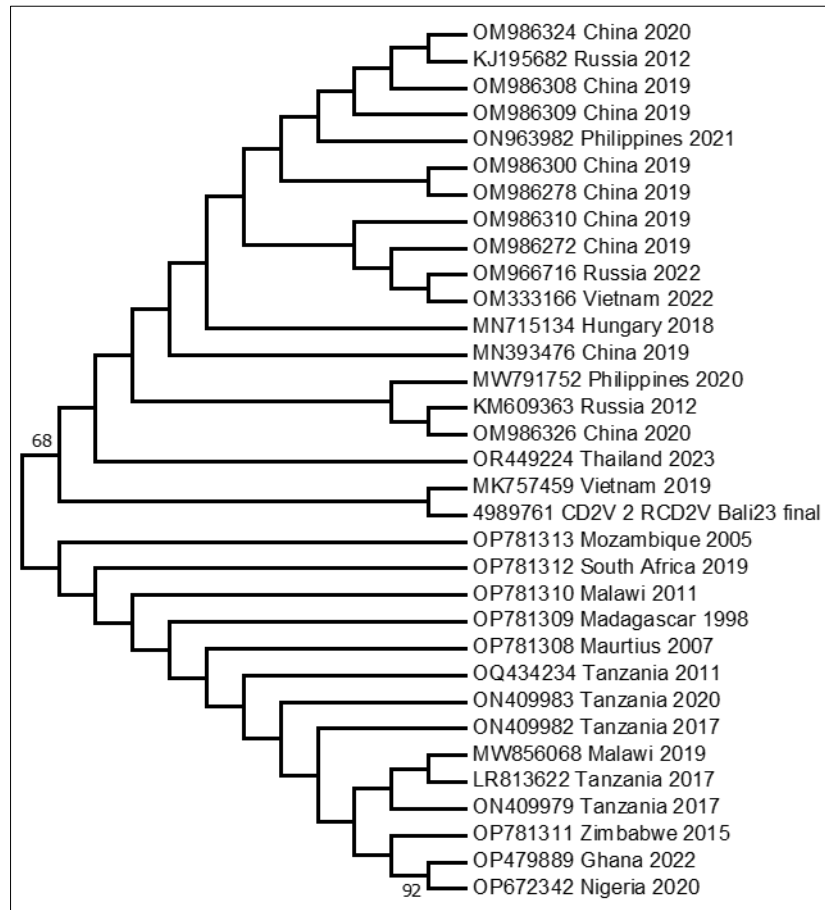


Figure 2 Molecular phylogenetic analysis result of CD2v gene fragment using Maximum Likelihood Tree method based on Kimura 2 model parameter. The accession number, country of origin and year of isolation are shown in the taxon names

In this study, DNA of ASF virus was obtained from an infected sample and two gene fragments, p72 and CD2v, were amplified with PCR and sequenced. The sequencing result of gene fragment p72 showed a total of 1842 out of 1942 base pairs. While for gene fragment CD2v, a total of 967 out of 1083 base pairs were sequenced.

Gene fragment p72 also annotated B646L, has a total of 1942 bp, coding the most dominant structural protein on the capsid (Miao et al., 2023). The results on genetic distance analysis between Bali/2023 virus and Cina/2019, Thailand/2023, Georgia, Mongolia/2019 and Vietnam/2022 showed a value of 0.001. The small value suggests that Bali/2023 is genetically very close or similar to the viruses found in other parts of Asia. On the other hand, the highest value for genetic distance is between Bali/2023 virus and ASF virus found in Cameroon, Italy, China/2022, and Mongolia/2022, which is possible because these viruses belong to a different genotype.

Nucleotide polymorphism analysis result is that the gene fragment p72 of Bali/2023 virus has an almost exact sequence to other viruses found in Asia such as China/2019 and Thailand/2023. When compared to other viruses, especially those with greater genetic distance, more polymorphic sites were found. Based on its nucleotide polymorphism, the p72 gene fragment is deemed to have significant genetic variation and thus used to determine the genotype of an ASF virus (Gallardo et al., 2023).

On amino acid level, the p72 gene of Bali/2023 virus has one polymorphic site when compared to China/2019 virus, where Bali/2023 virus has amino acid arginine (R) instead of serine (S) on amino acid number 326. Analysis on the entire data showed a total of seven polymorphic sites, which is significantly fewer than the number of nucleotide polymorphic sites (71). This suggests that many of the mutations in the p72 are silent mutation, where mutation on the nucleotide level does not cause changes in the amino acid sequence.

Phylogenetic analysis on gene fragment p72 is the foundation to determine the genotype of an ASF virus. The result (Figure 1) clearly shows that the Bali/2023 virus belongs in the same cluster with China/2019, Thailand/2022 and Mongolia/2019 which are all genotype II viruses. Therefore, it can be concluded that Bali/2023 virus belongs to genotype II.

Gene fragment CD2v (EP402R) has a sequence of 1083 bp, encoding protein that has significant roles in immune evasion and infection process (Li et al., 2023). This protein is also important in erythrocyte binding, together with other proteins found on the membrane such as p54, p22 and p12 (Vlasova et al., 2012). As for fragment p72, the same four genetic analyses were done on fragment CD2v. The result of genetic distance analysis shows that Bali/2023 has the exact same sequence with viruses found in Asia, namely China 2019 and Philippines/2021 as genetic distance is 0.000. Highest value for genetic distance is 0.003, the small number indicates that CD2v fragment has fewer genetic variations compared to p72 fragment (Le et al., 2019).

Phylogenetic analysis, unfortunately, did not form clear clusters for determining serogroup of the Bali/2023 virus. However, from the result obtained it can be seen that Bali/2023 virus is very closely related to Vietnam/2019 virus that belongs to serogroup 8 (Le et al., 2019).

For future studies, a few things that can be improved is the use of Next Generation Sequencing (NGS) to overcome the limitations of Sanger methods. More samples should also be analyzed, from different provinces in Indonesia for phylogeographic study, which will be useful for finding out about the virus origin, genetic variation and evolution. Phylogeography will give further understanding on how ASF entered Indonesia and its spread within the country. Based on the genetic analyses on gene fragments p72 and CD2v of Bali/2023 virus, it can be concluded that Bali/2023 ASF virus belongs to genotype II serogroup 8.

4. Conclusion

Molecular phylogenetic analysis on fragment p72 clearly placed Bali/2023 virus in genotype II cluster, while analysis on CD2v fragment placed Bali/2023 virus closest to Vietnam/2019 that belongs to serogroup 8.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Data Availability Statement

Data for genetic analyses results shown in this article is only part of the complete set, complete data is available upon request.

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