# WHOLE-GENOME SEQUENCING OF AFRICAN SWINE FEVER VIRUS IN SERBIAN SAMPLES 2019–2023

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## 1 Introduction

African swine fever (ASF) is a highly fatal haemorrhagic viral disease with a devastating economic impact on global pig production. In Serbia, ASF was first detected in domestic pigs in 2019, followed by a re-emergence in wild boars in 2020, where it has persisted. The aim of this study was to assess genomic changes of the ASF virus by performing shotgun metagenome sequencing on wild boar and domestic pig samples.

Summary/Key Points:

- Whole genome sequences were assembled and analysed to investigate phylogenetic relationships and identify mutations.
- The results showed the formation of a distinct Serbian subgroup and both major and minor genomic changes.
- These results emphasise the ongoing evolution of ASFV in Serbia and the crucial importance of continuous genomic surveillance.

## 2 Methods

This study utilised spleen samples from hunted or found dead wild boar, and dead domestic pigs. The samples were homogenized in a 1:10 ratio with PBS, centrifuged at 4000 rpm for 5 minutes, and the resulting supernatant was decanted and stored at -20 °C until further use. Viral DNA was extracted using the Indical IndiSpin Pathogen Kit (Indical, Germany). Five samples were selected for whole-genome sequencing based on the concentration and purity of extracted nucleic acids and sequenced on the Illumina platform. Whole genomes were assembled using Geneious Prime software, and phylogenetic analysis was performed with MEGA X software.

## 3 Results

Five high-quality genomes were obtained and deposited in NCBI GenBank (accession numbers OR660695–OR660699). Phylogenetic analysis, incorporating seven additional genomes, revealed three subgroups: strains NC044959, ON108571, and LR722599 formed the first subgroup; MT847622, MT847623, and LR899193 formed the second; while sequences from this study clustered with MK543947 in the third subgroup. Mutational analysis revealed both major and minor mutations. Major mutations contributed to enhanced strain classification and potential impacts on protein functionality. In contrast, minor mutations included extended poly-C and poly-G regions in MGF 110 and 360 gene families and ACD genes, as well as single deletions in intergenic regions. Silent mutations and poly-C/G insertions, although functionally unclear, were also observed.

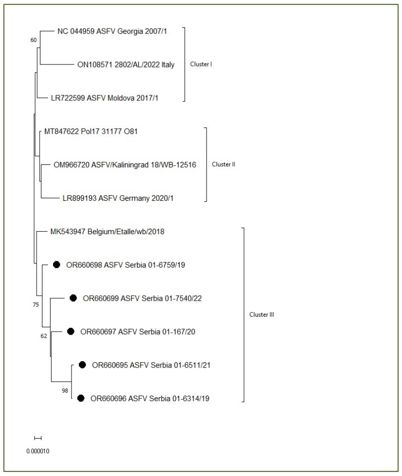

Figure 1: Phylogenetic tree of ASFV whole genomes by the ML method and the Tamura-Nei model.

## 4 Discussion

This study provides new insights into the genetic diversity of African swine fever virus (ASFV) in Serbia. Phylogenetic analysis of five ASFV genomes revealed three distinct subgroups, with sequences from this study clustering with MK543947. Major mutations helped to classify the strains, while minor changes such as poly C/G expansions and silent mutations indicated ongoing viral microevolution. Although their functional impact remains unclear, these results emphasise the ability of the virus to adapt and survive. This research presents critical insights into ASF virus genetic diversity and evolutionary dynamics in Serbia, contributing to the global understanding of its transmission and persistence. These findings underscore the importance of genomic surveillance in controlling this devastating disease.

## 5 Conclusion

- Five ASFV genomes were sequenced and deposited in GenBank.
- Phylogenetic analysis identified three subgroups of ASFV circulating strains.
- Mutations indicate a continuous ASFV evolution in Serbia.
- Continuous genomic surveillance is essential for ASFV control.

## 6 References & Aknowledgements

1. Gallardo C, Casado N, Soler A, et al (2023) A multi gene-approach genotyping method identifies 24 genetic clusters within the genotype II-European African swine fever viruses circulating from 2007 to 2022. Front Vet Sci 10:1112850. https://doi.org/10.3389/fvets.2023.1112850
2. Forth JH, Calvelage S, Fischer M, et al (2023) African swine fever virus – variants on the rise. Emerg Microbes Infect 12:2146537. https://doi.org/10.1080/22221751.2022.2146537
3. Milićević V, Kureljušić B, Maksimović Zorić J, et al (2019) First Occurence of African Swine Fever in Serbia. Acta Vet Brno 69:443–449. https://doi.org/10.2478/acve-2019-0038

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