WHOLE GENOME SEQUENCING OF WEST NILE VIRUS STRAINS FROM SERBIA

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Background

West Nile virus (WNV), family *Flaviviridae*, genus *Flavivirus*, is an arbovirus transmitted by mosquitoes of the genus *Culex*. The virus is maintained in the enzootic cycle with birds serving as amplifying hosts. WNV can infect a wide range of vertebrates, with humans and horses being particularly prone to clinical disease. The WNV genome consists of a single-stranded RNA molecule that encodes a polyprotein, which is cleaved into three structural and seven non-structural proteins. There are nine identified lineages, with lineages 1 and 2 being the most prevalent globally. Lineage 2 is predominant in Europe, causing the majority of human and animal cases. In Serbia, lineage 2 was first confirmed in 2010, and since then it has been detected in mosquitos, humans, birds, and horses.

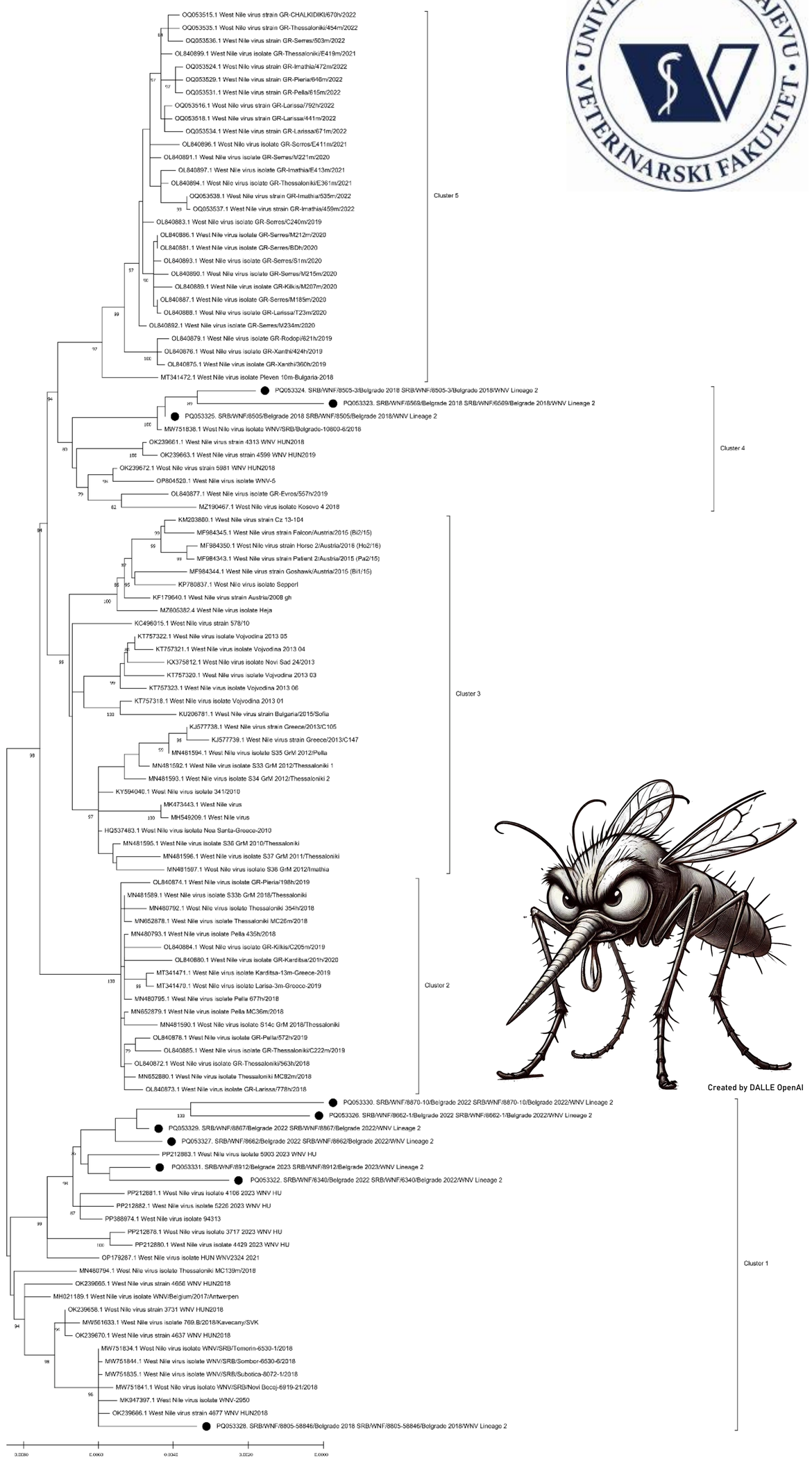
This study used the Oxford Nanopore platform to obtain and analyze whole genome sequences (WGS) of WNV strains from Serbia.

Methods

Samples used in this study were collected as part of an integrated national surveillance program for WNV. Positive RT-qPCR samples with CT value below 25 were subjected to WGS. For WGS 12 samples were selected, one mosquito and 11 cloacal swabs from birds. Sequencing was performed on the Oxford Nanopore MinION Mk1C device using targeted approach with 18 primer pairs generating 552 bp amplicons. Sequences were assembled using an in-house bioinformatic pipeline that utilizes a reference-based approach. Phylogenetic analysis was conducted in MEGA11 software with obtained consensus sequences. WGS and subsequent data processing were supported by the MediLabSecure Project.

Results

Sequencing was terminated after 12 hours, with the average quality score of the reads was 14.78. High-quality sequences were obtained from 10 (83.3%) samples. Based on the BLAST similarity results the highest percent identity was recorded with lineage 2 sequences PP212881.1 and PP212882.1 (Hungary) with 99.49% and 99.51% respectively. The phylogenetic tree with five clusters was reconstructed using 110 WGS from NCBI.



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Conclusion

Due to climate change, WNV is increasingly expanding in Europe and is now considered endemic in Serbia. In our study, seven sequences were grouped into a cluster with isolates from previous outbreaks in Serbia, Hungary, and Greece. The remaining three sequences clustered with isolates from Serbia, Hungary, Kosovo, and Greece. The similarity between Serbian and Hungarian strains can be attributed to natural pathways, with the geographical spread of WNV in Europe linked to river basins and bird migration routes. WGS is crucial for determining the molecular characteristics of circulating strains, providing essential data for phylogenetic analysis and molecular epidemiology. By updating existing protocols, novel rapid sequencing methods can be developed, enabling the swift identification and classification of circulating strains. This approach enhances our ability to monitor and respond to emerging infectious diseases effectively.

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