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ALPHAFOLD 3-BASED STRUCTURAL ANNOTATION OF HASEKI TICK VIRUS NONSTRUCTURAL PROTEINS *

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Abstract

Haseki tick virus (HSTV) nonstructural proteins were analyzed using AlphaFold 3 revealing structural similarity to *Orthoflavivirus*. NS3 helicase, NS3 protease, NS5 RNA-polymerase, and NS5 methyltransferase structures support taxonomic classification and antiviral drug development for this emerging tick-borne pathogen.

Haseki tick virus (HSTV) is a recently discovered tick-borne unclassified positive-sense single-stranded RNA virus first identified in blood sera from tick-bitten patients and Ixodes persulcatus ticks across Russian Federation regions in 2019 [1]. HSTV-infected patients exhibited fever and elevated temperature, suggesting pathogenic potential. Yet structural and functional data for HSTV proteins were lacking, impeding classification and drug development.

This study employed AlphaFold 3 neural network and bioinformatics approaches to predict tertiary structures and annotate functions of HSTV nonstructural proteins based on the principle that protein structure dictates molecular function [2]. The HSTV genome encodes an approximately 5100 amino acid polyprotein, and we analyzed the C-terminal nonstructural region containing essential viral replication machinery components.

Structural modeling identified four major nonstructural proteins with high confidence scores. HSTV NS3 protease (204 amino acids, 22 kDa, pLDDT 70.40) exhibited a catalytic triad topologically identical to *Orthoflavivirus* NS3 proteases, with highest structural similarity to Dengue virus 2 NS3 protease (TM score 0.79, RMSD 2.33 Å). The protein adopts dual β-barrel architecture with D1 and D2 domains connected by flexible linkers.

HSTV NS3 helicase (490 amino acids, 55 kDa, pLDDT 75.80) displayed superfamily 2 helicase architecture with three domains (D1-D3) and eight conserved structural motifs (I, Ia, II, III, IV, IVa, V, VI) essential for function. The protein showed highest structural similarity to Hepatitis C virus NS3 helicase (TM score 0.66, RMSD 3.46 Å). Biomolecular complex modeling with ATP and 50-nucleotide RNA confirmed functional RNA-binding capacity.

HSTV NS5 RNA-dependent RNA polymerase (RdRp) (711 amino acids, 82 kDa, pLDDT 87.41) adopted characteristic right-hand architecture with palm, finger, and thumb domains surrounding the active site. Highest structural similarity was observed with Dengue virus 2 NS5 RdRp (TM score 0.72, RMSD 3.26 Å). All catalytic motifs (A-G) were identified, which is critical for catalytic activity. The protein exhibited unique structural features including additional α-helices extending from the priming loop, potentially enhancing primer-independent polymerase function.

HSTV NS5 methyltransferase (MT) (231 amino acids, 26 kDa, pLDDT 71.10) showed classical α/β/α topology with highest structural similarity to *Pyrococcus horikoshii* MT (TM score 0.77, RMSD 3.02 Å). Notably, this domain was separated from NS5 RdRp by an unknown 323 amino acid domain (NS5-X), unusual for *Orthoflavivirus* genus members where MT and RdRp form continuous NS5 protein. Complex modeling revealed that NS5-X adopts structured conformation only when associated with both MT and RdRp domains, potentially representing novel functional arrangement for RNA capping machinery.

An analysis of the HSTV polyprotein also identified two putative transmembrane proteins, NSTR1 and NSTR2, each containing four membrane-spanning helices. However, we were unable to generate reliable tertiary models for NSTR1 and NSTR2 using AlphaFold 3, leaving their three-dimensional organization unresolved.

Structural alignments consistently showed highest similarity to *Orthoflavivirus* proteins, despite extremely low amino acid sequence identity below 30 percent. This structural conservation suggests evolutionary relationship and supports potential taxonomic classification of HSTV within *Orthoflavivirus* genus.

The identified structural similarities provide foundation for rational drug design targeting HSTV replication machinery. Conserved active sites in NS3 protease and helicase, along with NS5 polymerase catalytic center, represent potential therapeutic targets. Additionally, structural models enable development of diagnostic assays and vaccine antigens for this emerging tick-borne pathogen.

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These findings demonstrate AlphaFold 3 capabilities for functional annotation of non-homologous viral genomes, revealing hidden structural relationships despite sequence divergence. Understanding HSTV structural biology is vital for readiness against emerging viral threats.

References

1. Kartashov M. Y., Gladysheva A. V., Shvalov A. N. et al. Novel Flavi-like virus in ixodid ticks and patients in Russia // Ticks Tick Borne Dis. 2023.

2. Gladysheva A. V., Osinkina I. A., Radchenko N. S. et al. Tertiary Structures of Haseki Tick Virus Nonstructural Proteins Are Similar to Those of Orthoflaviviruses // Int. J. Mol. Sci. 2024.