



# Full- genome analysis of the influenza A (H1N1) pdm09 isolated in Ukraine during 2009-2017

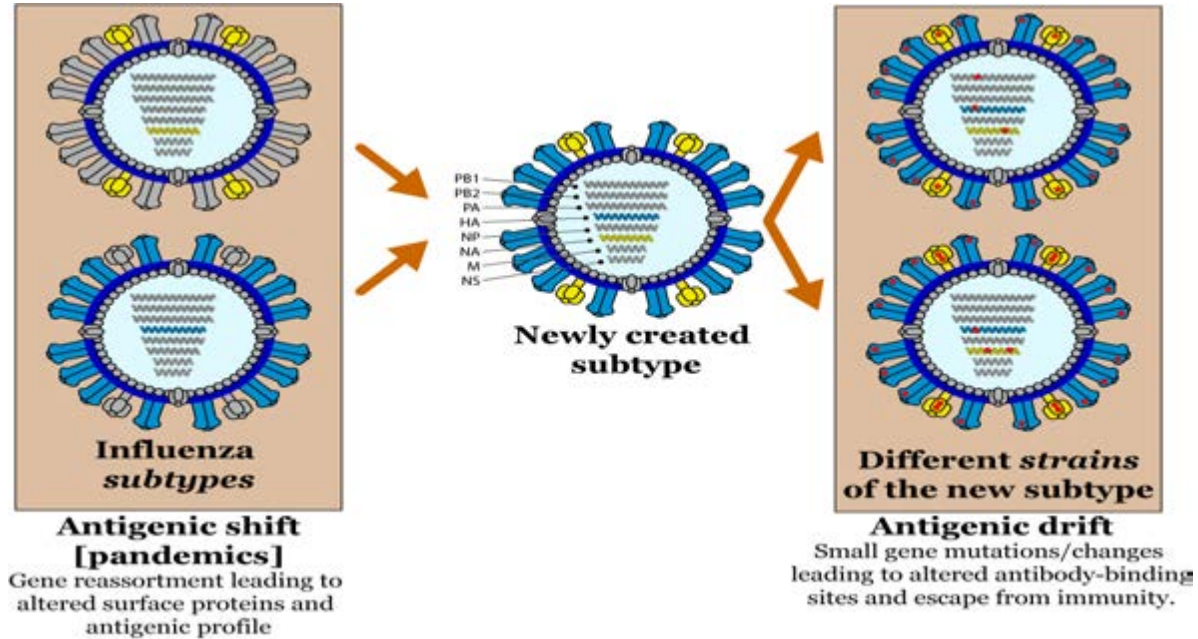
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# Relevance



<https://virologydownunder.blogspot.com/2013/07/influenza-viruses-ifvs.html>

# Study Purpose

- The main aim of this study was to carry out the molecular-genetic and phylogenetic analysis of all genes of pandemic A(H1N1) influenza virus, which circulated in Ukraine during 2009-2017 to determine the genetic characteristics related to clades, antiviral resistance, virulence, pathogenicity, and polymerase activity.

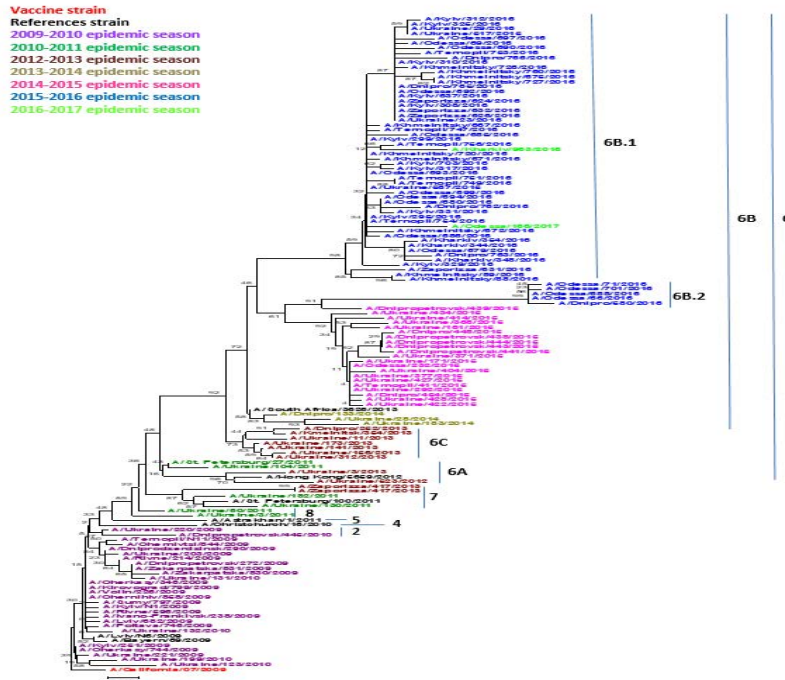
# Methods

- Samples taken from patients from the polyclinic of sentinel cities of Ukraine;
- Extraction of the nucleic acid of influenza viruses (were used extraction kit - InnuPREP Virus DNA/RNA);
- Real-time polymerase chain reaction (RT-PCR) was carried out by the protocol given by WHO, 2009 (Termo Scientific Verso 1-Step qRT-PCR ROX Kit) ;
- Isolation and cultivation of influenza viruses was carried in MDCK cell (epithelial cells kidneys female Cocker -Spaniel);
- Sequencing (the sequences of viruses from other countries were received from web-site GISAID using BLAST analysis);
- Phylogenetic analysis (MEGA7 software);
- Molecular-genetic analysis;
- Construction of structural 3D models (Chimera 1.11.2rc software).

# Influenza surveillance centers in Ukraine



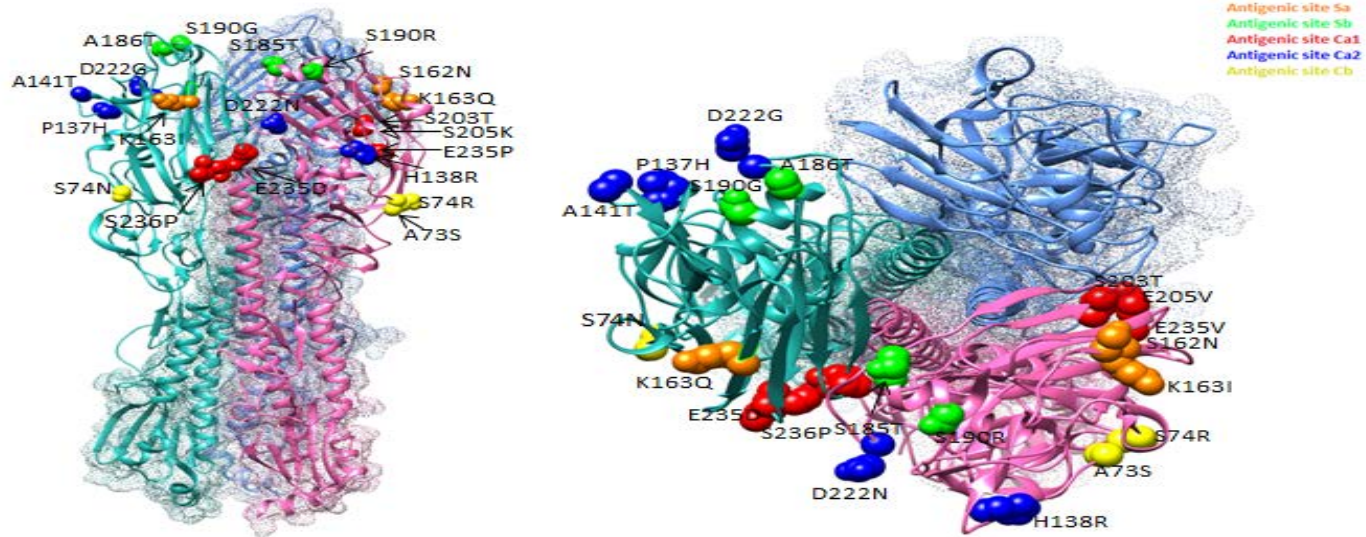
# The distribution of viruses A(H1N1)pdm09 among genetic groups from 2009 to 2017



- During 2009–2017 the gradual genetic alterations occurred, which led to the virus antigenic property changes.
- The viruses belong to 8 genetic groups that circulated in different epidemic seasons in the world. Ukrainian isolates belong to 2, 6, 7 and 8 genetic groups in this period.
- Starting from 2010-2011, influenza viruses of group 6 were predominantly circulating not only in Ukraine, but also throughout the world.
- The group 6 of viruses is divided into 6A, 6B and 6C. Starting from 2015-2016, the viruses of the genetic group 6B were subdivided into subgroups 6B.1 and 6B.2.

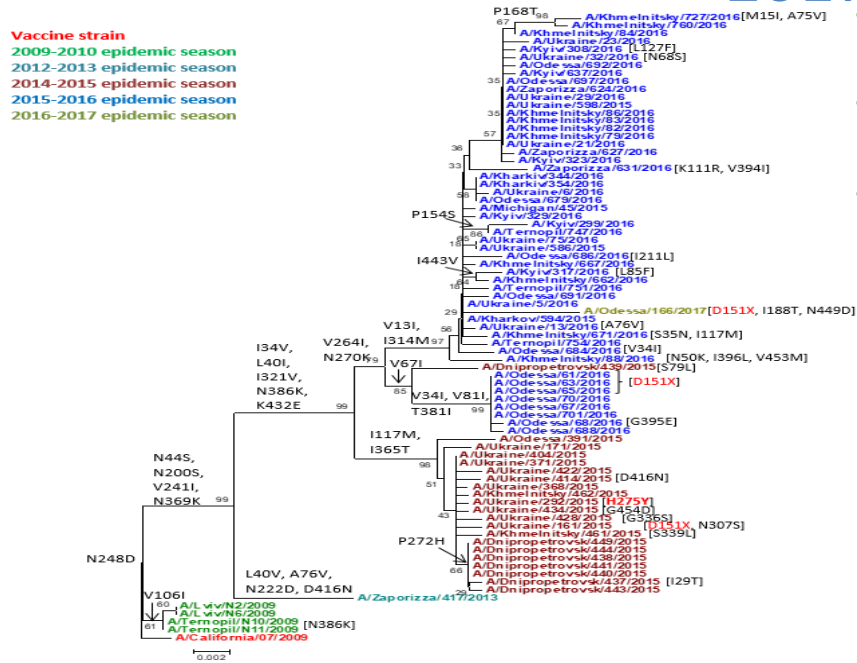
Phylogenetic tree of HA gene A(H1N1)pdm09 viruses

# Amino acid substitutes in antigenic sites of HA influenza A (H1N1) pdm09 viruses detected among isolates in Ukraine from 2009 to 2017



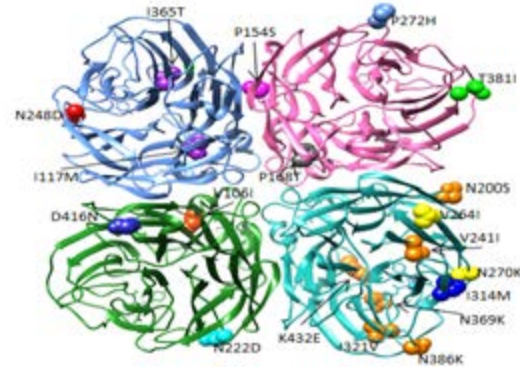
Localization of mutations in HA (PDB ID:3lzg)

# Molecular-genetic analysis of NA A(H1N1)pdm09 viruses isolated during 2009-2017



Phylogenetic tree of NA gene A(H1N1)pdm09 viruses

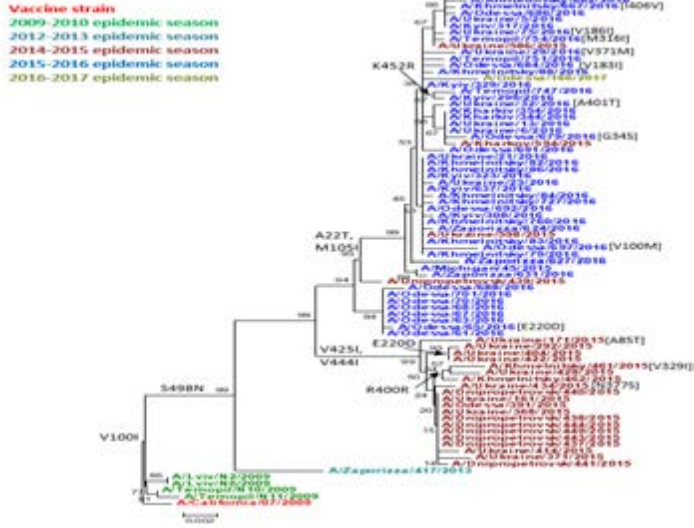
- Viruses isolated during 2009–2017, belong to different antigenic groups.
- Most of the isolates for the neuraminidase gene belong to the genetic group 6B.
- In the NA genes were observed mutations H275Y and D151X causing resistance to antiviral drugs in different years.



Localization of mutations in NA (PDB ID:3nss)

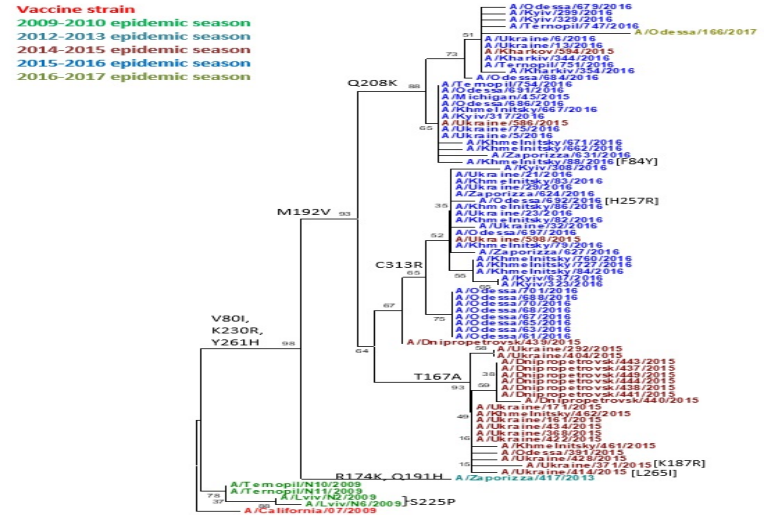


# Molecular-genetic analysis of NP and MP A(H1N1)pdm09 viruses isolated during 2009-2017



Phylogenetic tree of NP gene A(H1N1)pdm09 viruses

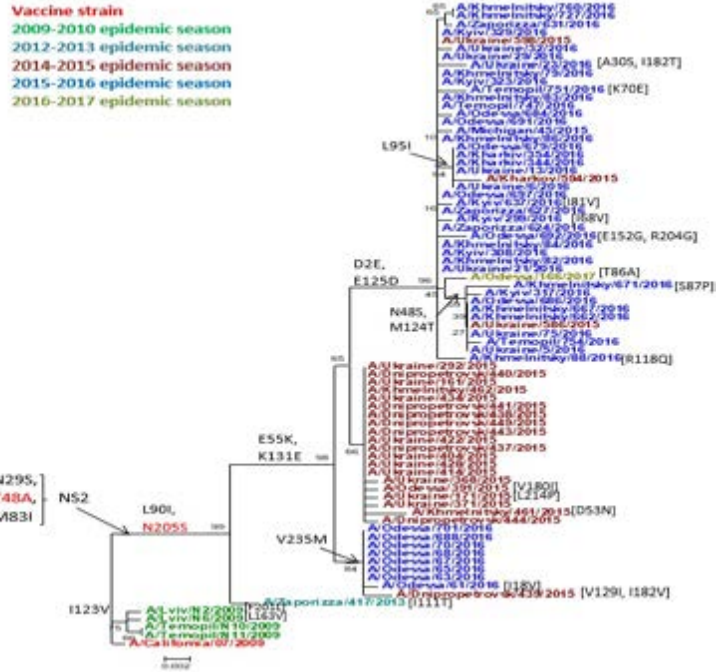
- NP genes had mutations N377S and V425I, which can influence the immune response.
- In 2015-2016, in NP genes emerged two mutations A22T and M105I.



Phylogenetic tree of MP gene A(H1N1)pdm09 viruses

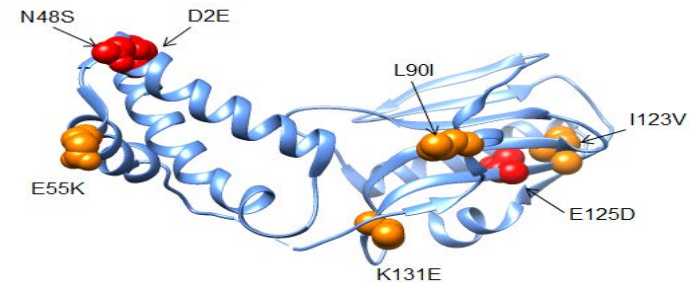
- In M1 were observed numerous mutations, the functional value of most mutations is unknown. However, F84Y mutation can influence the immune response.
- All isolates including the A/California/07/2009 vaccine strain had the D21G mutation in the M2 protein, causing resistance to antiviral drugs such as amantadine and rimantadine.

# Molecular-genetic analysis of NS A(H1N1)pdm09 viruses isolated during 2009-2017



Phylogenetic tree of NS gene A(H1N1)pdm09 viruses

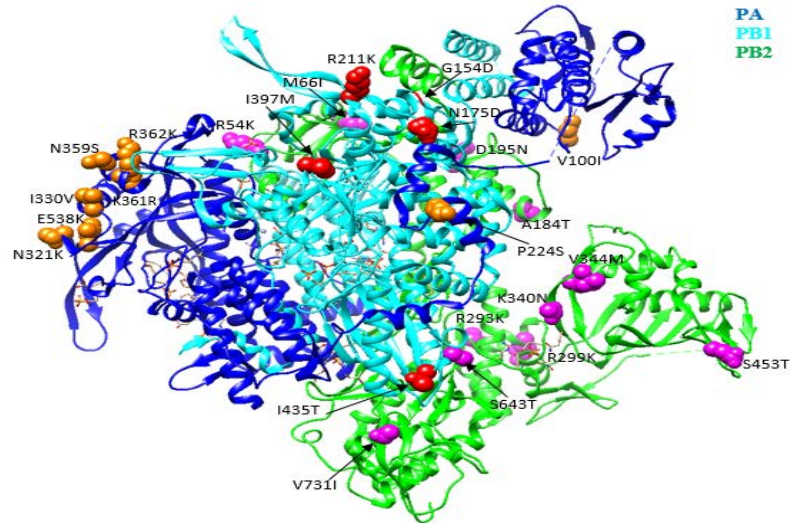
- Viral NS1 protein is the main factor of virulence.
- In 2015-2016 epidemic season there were identified amino acid substitutions D2E, N48S, and E125D in the NS1 protein of Ukrainian isolates. These mutations cause the increase in virulence of the virus and can inhibit human immune response.



Localization of mutations in NS (PDB ID:3f5t)

# Molecular-genetic analysis of polymerase complex A(H1N1)pdm09 viruses isolated during 2009-2017

- The viruses were located similarly on PA, PB1, PB2 phylogenetic trees.
- In PA there was P560S mutation that increase virulence. In addition, we found unique changes in all genes of the polymerase complex in different epidemic seasons PA – T162I, C241Y, A448T, T528I; PB1 – R190K, P454S, S515P; PB2 – E72G, R299K, M365L. The role of these mutations is unknown.
- One isolate A/Odessa/692/2016 had mutation R591X in PB2, which according to genetic studies leads to increased virulence of the virus in mice. Also, this substitution plays a role in binding alpha importin of host.



Localization of mutations in Pol (PDB ID:4wsb)

# Conclusions

- Ukrainian isolates on HA tree belonged to 2, 6, 7 and 8 genetic groups in period from 2009 to 2017.
- Molecular genetic changes occurred in each of the five antigenic sites of the HA influenza A (H1N1) pdm09 viruses.
- In NA genes were observed H275Y and D151X mutations that caused resistance to antiviral drugs in different years.
- NS1 genes had few mutations, namely E55E, E125D and N205S, which can inhibit human immune response and cause the increase of the viral virulence.
- All viruses had mutation D21G in M2, which causes resistance to antiviral drugs such as amantadine and rimantadine.
- In Pol genes were observed numerous mutations, which according to the literature review , in combination may lead to increased virulence of the pandemic A(H1N1)pdm09 influenza viruses (PA there was P560S, R591X in PB2) .

# Acknowledgment

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- The authors would like to acknowledge the WHO Collaborating Centre for the Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention (Atlanta, United States of America) for their collaboration on influenza surveillance and sequencing of viruses.

Thank you for  
your attention!