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GENETIC MONITORING OF CIRCULATING INFLUENZA VIRUSES IN UKRAINE DURING 2010-2011 INFLUENZA SEASON

Результаты филогенетичского анализа украинских изолятов вируса гриппа эпидемического сезона 2010-2011 показали их схожесть с таковыми в мире. Анализ мутаций обнаружил важные аминокислотные замены в генах гемагглютинина украинских изолятов.

The phylogenetic analysis of Ukrainian influenza viruses during 2010-2011 epidemic season showed their similarity to flu isolates all around the world. Mutation finding indicated significant amino acid substitution in the hemagglutinin genes of Ukrainian viruses.

Annual influenza epidemics in many countries, considerably in Northern Hemisphere, are caused by continuous variability of influenza viruses and extreme activity of their transmission. In fact, epidemic season 2010-2011 has lower intensity and les number of selected viruses from the previous 2009-2010 pandemic season.

Monitoring the genetic changes of influenza viruses appeared in our country not long ago, but has already proved its relevance and necessity. Such data can considerable speed up the adequate response to new or changed viruses. So, the work of all public health services in country was adjusted, after confirmating first selected pandemic A(H1N1) sw influenza isolate.

The aim of our study was to perform the genetic monitoring of influenza viruses isolated in Ukraine and to compare with viruses from other countries which circulated during the 2010-2011 epidemic season by constructing the phylogenetic trees.

Materials and methods. Nasopharyngeal swabs and autopsy materials collected from infected patients, were received during the epidemic from different regions of Ukraine. Typing of viruses was performed by real-time RT-PCR (reverse transcription polymerase latsyuhova reaction in real time). Strain definition was performed with using the diagnostic serum sent by Center for Disease Control and Prevention (CDC Atlanta, USA). For genetic analysis were used sequences of hemagglutinin gene of Ukrainian influenza viruses A(H1N1)sw, A(H3N2) and B. The GISAID web resource [1] was used for searching sequences essential for the analysis. The identification and comparison of obtained sequences was performed in BLAST (Basic Local Aligment Search Tool) [2]. Phylogenetic comparison and constructing the phylogenetic trees was performed in MEGA 5 program software [3] with using the Neighbor-Joining (combining nearest neighbors) [4] method. The aligning of sequences was performed with using ClustalW algorithm [5]. For the statistical evaluation of the results significance was used Bootstrap analysis with the number of replications 1000 [6].

Results and discussion. Influenza season 2010-2011 was the first after the pandemic season in Ukraine. The distinction of this season was early wide circulation of influenza B viruses (autumn 2010) and ongoing circulation of pandemic A(H1N1)sw influenza viruses (from the second half of January and until the beginning of April). And only two of A(H3N2) influenza viruses have been isolated. Genetic analysis was directed to hemagglutinin genes as those with high level of variability. More over the hemagglutinin genes are the primary target for neutralizing antibodies.

Phylogenetic comparison of hemagglutinin (HA) genes of influenza Asw (H1N1) viruses

The pandemic A (H1N1) sw influenza viruses continued to be isolated in the 2010-2011 epidemic season despite their appearence in early 2009 by triple gene recombination of human, swine and bird influenza viruses [7]. It is known from the literature that hemagglutinin of pandemic influenza viruses has classical swine flu virus derivation (like ones, caused the pandemic of 1918-1919), which distinguishes them from human or birds influenza viruses [8].

We began to research the hemagglutinin sequences of Ukrainian isolates from finding similar genetic sequences with using BLAST search too. So, we found viruses with high percentages of similarity for the hemagglutinin genes: isolate from a 36 years old man, who has ill after contact with a sick animal – A/Ohio/01/2007 (the similarity 93%), and swine influenza virus – A/swine/Illinois/1/1975 (similarity – 87%). Also in analysis were taken the reference strain of seasonal human influenza virus (H1N1) – A/New Caledonia/20/1999 with 7% similarity (Fig. 1.).

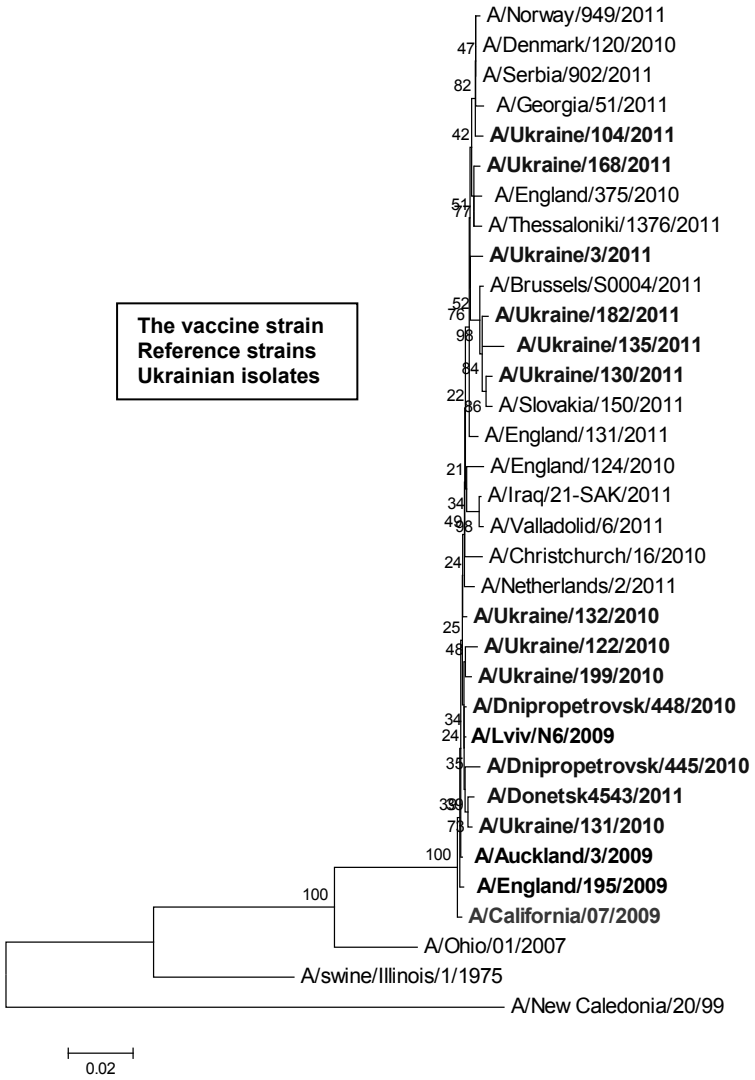

Fig.1. Phylogenetic tree of hemagglutinin (HA) gene sequences of influenza A(H1N1)viruses constructed by NJ method with Kimura 2-parameter model (Bootstrap values are indicated)

According to the results of Hemagglutinin inhibition (HI) assay our isolate A/Lviv/N6/2009 was selected as reference strain due to its low binding activity to ferret antisera comparing to the vaccine strain A/California/07/2009 (in titer of 160 against 1280).

Having completed all steps of analysis, we obtained phylogenetic tree where hemagglutinin genes of Ukrainian isolates comparably to viruses isolated in other countries, including vaccine and reference strains (Fig. 1.). We can see from the figure that viruses isolated in Ukraine in autumn 2010 placed at the bottom of phylogenetic tree near the reference strain A/Lviv/N6/2009. But isolates fron early 2011 had gained changes that determined them in another cluster. Also we have found one exception – A/Donetsk/4543/2011, which remained genetically similar to isolates from autumn 2010, although was isolated in 2011.

Also, for more detailed analysis of the hemagglutinin gene of pandemic influenza viruses, we constructed a phylogenetic tree, with only pandemic influenza viruses (Fig. 2.). More over, we find amino acid differences in sequences of hemagglutinin proteins of Ukrainian isolates in comparison with the vaccine and reference strains. Three isolates, A/Ukraine/130/2011, A/Ukraine/135/2011 and A/Ukraine/182/2011 formed a separate cluster with viruses isolated in Brussels and Slovakia according their differences. In hemagglutinin of these viruses were found following amino acid substitutions: S143G (glutamine changed serine at position 143) and A197T (threonine changed alanine at position 197).

In addition, isolate A/Ukraine/135/2011 showed polymorphism in the nucleotide sequence encoding the 155 amino acid residue of hemagglutinin protein. According to the World Influenza Centre (London) polymorphism or nucleotide substitution in the region, which encodes residues from 153 to 157 is often associated with low binding ability to antisera. It is interesting that the polymorphism in this region was not observed in clinical samples. It can be an artifact of reproduction in cell culture, especially for the MDCK-siat line, that ceased to use in study of influenza A(H1N1)sw viruses [9].

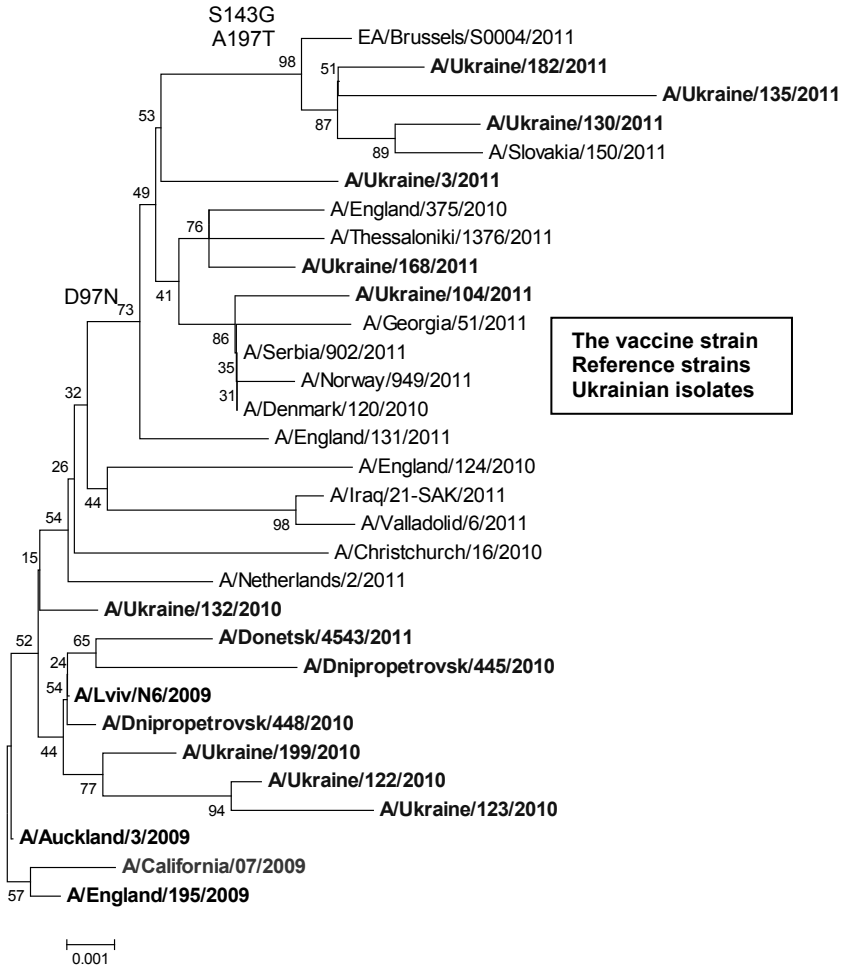

Fig. 2. Phylogenetic tree of hemagglutinin (HA) gene sequences of pandemic influenza A(H1N1)sw viruses constructed by NJ method with Kimura 2-parameter model (Bootstrap values are indicated)

All pandemic influenza viruses isolated in our country has preserved similarity to vaccine strain A/California/07/2009 near 98%, but showed minor changes in the hemagglutinin genes(Fig. 2.).

Phylogenetic comparison of hemagglutinin (HA) genes of influenza B viruses

All isolated and sequenced influenza viruses type B belonged to B/Victoria/2/87genetic branch that was dominating worldwide during this epidemic season (about 85%). As we can see from the phylogenetic tree (Fig. 3) all Ukrainian isolates (marked blue) were similar to the vaccine strain B/Brisbane/60/2008, except few mutations.

Amino acid substitutions in hemagglutinin of Ukrainian influenza B viruses: N75K (aspartic acid at position 75 of hemagglutinin protein is replaced by the lysine), N165K (aspartic acid is replaced by lysine at position 165) and S175P (serine replaced by proline at position 175) determined them into cluster B/Brisbane/60/2008-like viruses. And mutation that caused the L58P substitution (leucine replaced by proline at position 58) grouped all sequenced influenza viruses type B, isolated in Ukraine into single cluster, which included most of the selected isolates in this season (Fig.3.). Also Ukrainian isolate B/Odessa/3886/2010 was selected in London as the reference strain (Fig. 3.) among the viruses cultivated L58P substitution.

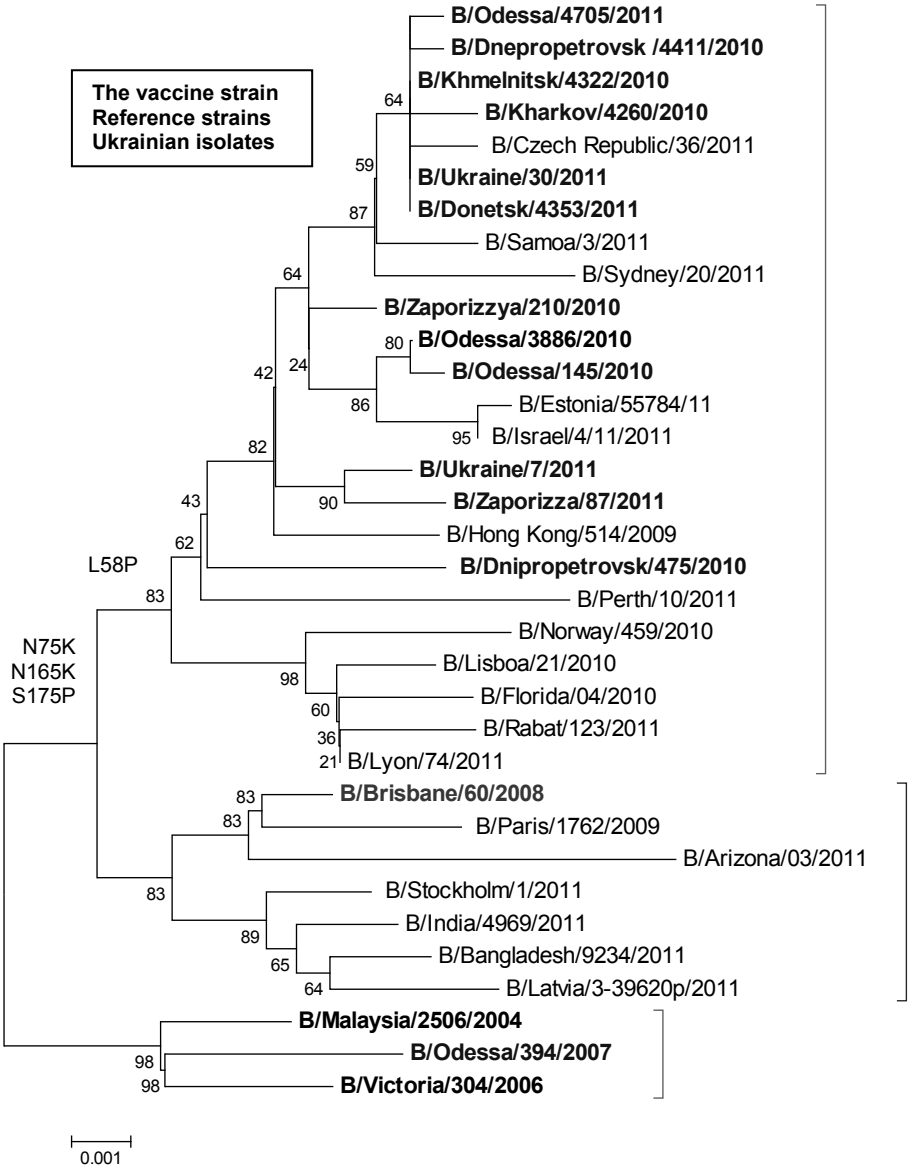

Fig. 3. Phylogenetic tree of hemagglutinin(HA) genes of influenza viruses type B B/Victoria/2/87 line constructed by NJ method with Kimura 2-parameter model (Bootstrap values are indicated)

Phylogenetic comparison of hemagglutinin (HA) genesofinfluenza A (H3N2) viruses

We have selected only two isolates belonged to A(H3N2) subtype:A/Khmelnitsky/1340/2010 and A/Ukraine/175/2011, isolated in our laboratory during the 2010-2011epidemic season. They amounted to 1% among the total number of influenza viruses isolated in Ukraine. This subtype of influenza A viruses was isolated in USA, Canada, Europe, Africa, Hong Kong and Argentina.

Phylogenetic analysis showed that virus isolated in Khmelnytsky has located near A/Brisbane/10/2007 and A/Wisconsin/67/2005 viruses – earlier representatives of this genetic branch. This mean that isolate was evolutionarily "old", and has preserved genetic similarity to viruses 2005 – 2007 years (Fig.4.). As for isolate A/Ukraine/175/2011, it located within A/Victoria/208/2009 cluster, which included the majority of influenza A(H3N2) viruses from this epidemic season. Isolate A/Ukraine/175/2011showed the greatest genetic similarity to viruses isolated in Brazil, Moldova, Russia, etc., which were joined into subclaster A/Pert/10/2010-like viruses (Fig. 4.).

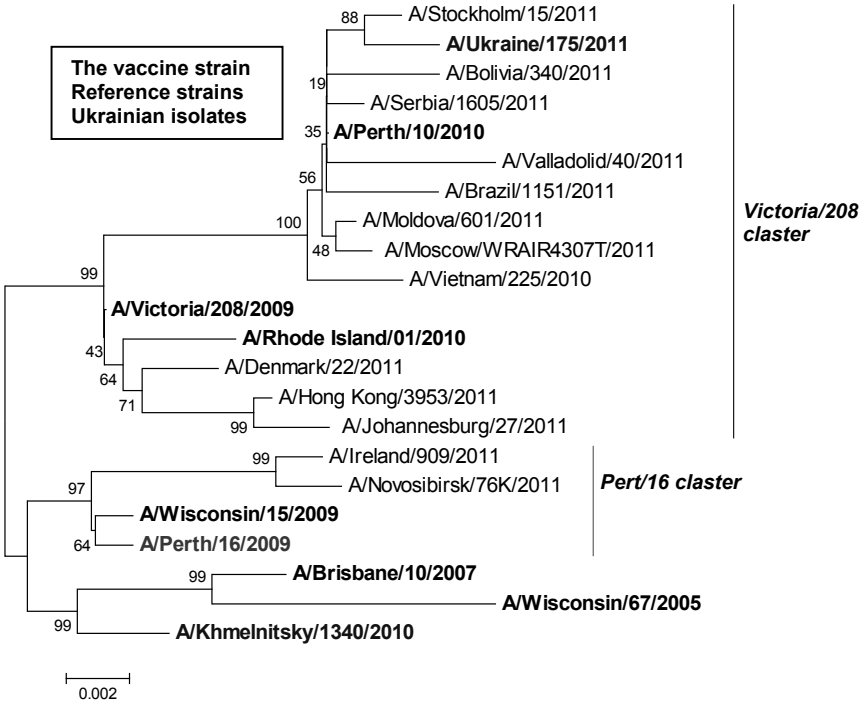

Fig. 4. Phylogenetic tree of hemagglutinin(HA) gene influenza A(H3N2)viruses constructed by NJ method with Kimura 2-parameter model (Bootstrap values are indicated)

Influenza A(H3N2)viruses, isolated during the 2010-2011 epidemic season in the world belonged to both Victoria and Pert clusters due to their genetic differences in the hemagglutinin. MutationsR261Q, E50K, P162S, N133D, R142G A212T and V213A were observed within A/Perth/16/2009 cluster, that differ viruses from A/Brisbane/10/2007. In cluster A/Victoria/208/2009 were observed next substitution in the hemagglutinin gene: N145S, V223I, N312S, D53N, Y94H, I230V, E280A and S312N (Fig.4.).

Hemagglutination inhibition assay showed that all Ukrainian isolates reacted in high enough titres with strainspecific serum to reference strains. It demonstrates their antigenic affinity to the vaccine strain A/Perth/16/2009.

Conclusions. The analysis of circulating influenza viruses in 2010-2011 epidemic season in Ukraine showed that epidemic was caused mainly by influenza viruses type B (up 55% of all selected viruses). Almost 45% of influenza isolates were similar to pandemic A(H1N1)sw strain. And only 2 isolates belonged to subtype A(H3N2).

Pandemic viruses A (H1N1)sw isolated in Ukraine were placed within two clusters, according their genetic structure. The amino acid substitutions N75K, N165K and S175P identified Ukrainian isolates type B as B/Brisbane/60/2008-like viruses. Phylogenetic analysis of influenza A(H3N2) viruses showed that isolate A/Khmelnitsky/1340/2010 located near A/Brisbane/10/2007 viruses – earlier representatives of this genetic branch. And virus A/Ukraine/175/2011 located within A/Victoria/208/2009 cluster, and showed the greatest genetic affinity to the A/Pert/10/2010-like viruses.All isolated in 2010-2011 influenza season viruses were antigenically related to vaccine strains.

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FEATURES OF POLYPEPTIDE COMPOSITION OF PHAGES ISOLATED FROM ANTARCTIC SAMPLES OF SOIL AND MOSS ON DIFFERENT BACTERIA STRAINS – *XANTHOMONAS AXONOPODIS PV. BETICOLA 7325* AND *ERWINIA CAROTOVORA 216*

Исследование морфологии и полипептидного состав фагов, выделенных из антарктических образцов почвы и мха на бактериях Xanthomonas axonopodis pv. beticola 7325 и Erwinia carotovora 216 IMV. Для исследуемых фагов, подобных морфологически, показана подобность и в полипептидном составе. Группа подобных фагов диференциировалась по составу минорных белков.

The morphology and polypeptide composition of phages isolated from Antarctic samples of soil and moss on bacteria X. axonopodis pv. beticola 7325 and E. carotovora 216 IMV were studied. For phages similar morphologically, similarities and polypeptide composition were shown. Group of similar phages differ in composition of minor proteins.

Introduction. Even though bacteriophages play a major role in maintaining ecological balance of microbial communities, their potential influence on biogeographical patterns of bacterial communities remain greatly under-investigated. Features of their evolutionary relationships, adaptation to