и асоциации Salicetum albae. Тополевые леса низовий поймы Сулы отнесены к союзу Calamagrostio epigei-Populion nigrae и распределяются на две ассоциации, название которых предлагаем изменить согласно требований Международного кодекса фитосоциологической номенклатуры – на Galio veri-Populetum nigrae и Strophiostomo sparsiflorae-Populetum albae. Указывается на отсутствие в исследуемых сообществах видов растений из Красной книги Украины. Наименее трансформированные, наибольшие по площади и более старые ольховые, ивовые и тополевые леса каждой из ассоциаций требуют заповедания, в пользу чего свидетельствует их значительная водоохранная роль.

Ключевые слова: Querco-Fagetea (Alno-Ulmion), Alnetea glutinosae, Populetea albae, Украина, Левобережная Лесостепь, бассейн нижней Сулы, синтаксономия.

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MOLECULAR AND GENETIC CHARACTERISTICS OF SURFACE AND NONSTRUCTURE PROTEINS OF PANDEMIC INFLUENZA VIRUSES A(H1N1)PDM09 IN 2015-2016 EPIDEMIC SEASON

The aim of the present study was identifying of molecular and genetic changes in hemaglutinin (HA), neuraminidase (NA) and non-structure protein (NS1) genes of pandemic influenza A(H1N1)pdm09 strains, that circulated in Ukraine during 2015-2016 epidemic season. Samples (nasopharyngeal swabs from patients) were analyzed using real-time polymerase chain reaction (RT-PCR). Phylogenetic trees were constructed using MEGA 7 software. 3D structures were constructed in Chimera 1.11.2rc software.Viruses were collected in 2015-2016 season fell into genetic group 6B and in two emerging subgroups, 6B.1 and 6B.2 by gene of HA and NA. Subgroups 6B.1 and 6B.2 are defined by the following amino acid substitutions. In the NS1 protein were identified new amino acid substitutions D2E, N48S, and E125D in 2015-2016 epidemic season. Specific changes were observed in HA protein antigenic sites, but viruses saved similarity to vaccine strain. NS1 protein acquired substitution associated with increased virulence of the influenza virus.

Key words: A(H1N1)pdm09 influenza virus, amino acid substitution, antigenic site, non-structure protein.

Introduction. Influenza viruses are antigenicaly variable pathogens, capable of continuously evading immune response. Accumulation of mutations in the antigenic sites is called the "antigenic drift". In circulating influenza viruses this antigenic drift is a major process, accumulating mutations at the antibody binding sites of receptor proteins, and enabling the virus to evade recognition by hosts' antibodies, which often translates into periodic epidemics of influenza. To tame the influenza spread a flexible vaccination WHO's program, based on periodic production of novel versions of vaccine, is adapted to the actually prevalent stain(s). For such programs the data on phylogenesis of circulating versions of pathogens, and genetic stability of their hemagglutinin (HA) sets data, could help to rationalize possible epidemiological measures [1].

This year's seasonal influenza risk assessment identifies type A viruses, in particular A(H1N1)pdm09, as dominant thus far in EU/EEA countries. There are strong indications from some EU/EEA countries that the A(H1N1)pdm09 virus is responsible for the hospitalization of a large number of severe cases. This includes hospitalizations for severe outcomes for both risk groups and otherwise healthy young adults. A similar pattern of severity is likely to be observed in other countries as the season progresses [2].

Matherials and methods. Samples were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses subtype A(H1N1)pdm09 were isolated in MDCK and MDCK-SIAT cell culture from samples positive in PCR. Hemagglutinin (HA), neuraminidase (NA) and non-structure protein (NS1) gene sequences of Ukrainian isolates were selected to perform phylogenetic comparisons. Phylogenetic analysis was performed using MEGA 7 software [3]. The influenza A(H1N1)pdm09 sequences are characterized in a neighbor-joining phylogenetic tree with reference strains rooted from the current vaccine strain, A/California/07/2009-like virus. 3D structures were constructed in Chimera 1.11.2rc software [4].

Results and discussion. In this study we compared nucleotide sequences of influenza viruses HA, NA and NS1 proteins.

Comparison of neuraminidase (HA) genes.

Over the last five years the HA genes have evolved and eight genetic groups have been designated, with A/California/7/2009 representing group 1, and viruses in group 6 have formed clusters designated groups 6A, 6B and 6C. Viruses collected in 2015-2016 season fell into genetic group 6B and in two emerging subgroups, 6B.1 and 6B.2. Subgroup 6B and subgroups 6B.1 and 6B.2 are defined by the following amino acid substitutions in HA1 and HA2.

Most of the viruses had amino acid substitutions that define the new group of viruses in genetic group 6B, now called group 6B.1. Isolates had a substitution at one of these sites N162K resulting in loss of glycosylation site, acquired by the 6B.1 viruses (fig.1).

Also Ukrainian viruses had substitutions S84N and I216T. Three isolates from Khmelnitsky, Kiev and Ternopol had unique mutation in HA2 – I91V. New substitution S83P was observed in the majority of viruses from 6B.1 group. Four isolates from Odessa belonged to group 6B.2. Its HA protein had a substitution at residue 152 of HA1, V152T. Substitutions in this region, as well as at residue 152, are often selected in culture and known to affect the antigenicity of the virus. Viruses from group 6B.2 also had substitutions R113K D127E (gain of glycosylation site) and E47Q (HA2).

Gain or loss of *N*-linked glycosylation sites has been shown to alter HA protein surface topology. A gain in glycosylation could be advantageous to the virus by virtue of a masking effect on important antibody recognition sites, thus potentially modulating viral antigenicity [5]. Observations are based solely on sequence motifs. For the influenza A(H1N1)pdm09 specimens characterized in this report, two mutations, S162N (serine to asparagine) and D127E (asparagine acid to glycine acid), were observed that could cause a gain of a glycosylation motif.

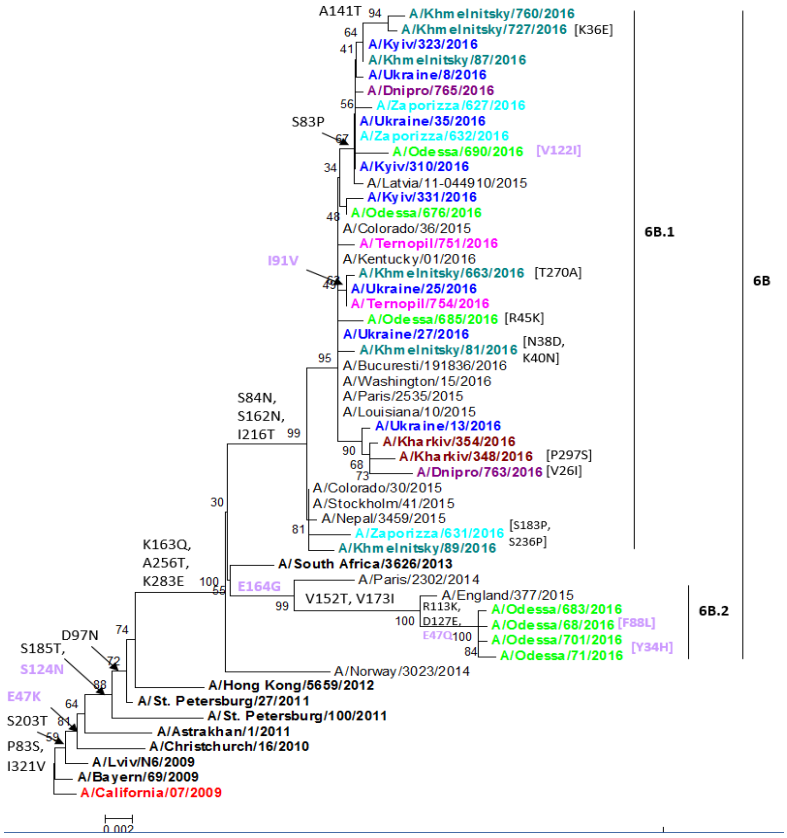

Fig.1. Phylogenetic tree of the HA gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

It is known that the H1 HA molecules have four distinct antigenic sites: Sa, Sb, Ca, and Cb [6]. As a result, these sites consist of the most variable amino acids in the HA molecule of the seasonal human H1N1 viruses that have been subjected to antibody-mediated immune pressure. Notably, the Sa and Sb sites that contain many amino acids involved in neutralizing epitopes near the receptor binding pockets [6].

In Ukrainian isolates were observed mutations in antigenic sites, which emerged in 2015-2016 epidemic season. The main substitution S162N emerged in Sa antigenic site and was observed in all isolates from group 6B.1 (fig.2).

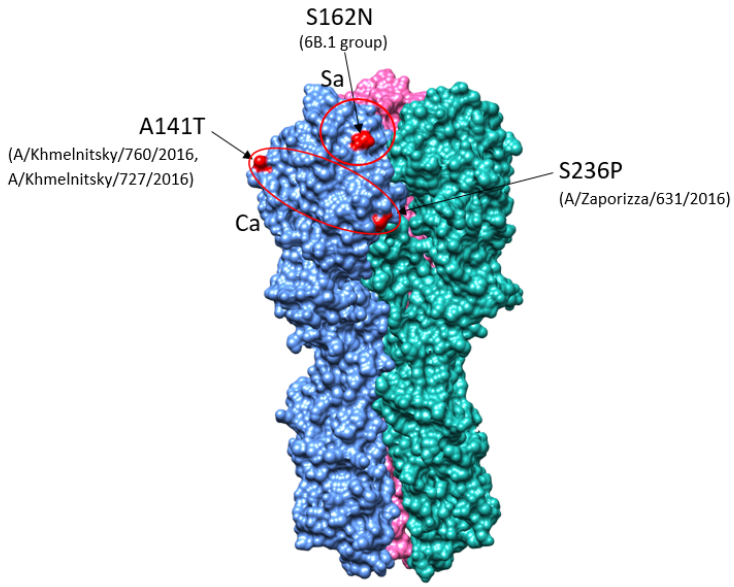

Fig.2. 3D structure of antigenic sites on the HA molecule of Ukrainian isolates

Two substitutions were observed in antigenic site Ca, A141T – had isolates №727 and №760 from Khmelnitsky, S236P – in A/Zaporizza/631/2016. Information about changes in antigenic sites very important for prediction next dominant strains. It is well-documented that antigenic changes of HA occasionally result in the acquisition of carbohydrate side chains on the HA molecule [7]. Since the carbohydrate side chains in the vicinity of antigenic sites mask the neutralizing

epitopes on the HA surface, amino acid substitutions associated with acquisition of carbohydrate chains are believed to efficiently generate antigenic variants.

Comparison of neuraminidase (NA) genes.

Genetic comparison of influenza virus A(H1N1)pdm neuraminidase genes shown that all investigated isolates were genetically related to reference strain A/South Africa/3626/2013 and saved high genetic similarity to vaccines strain A/California/07/2009. On phylogenetic tree of NA genes was shown that viruses also divided into two subgroups – 6B.1 and 6B.2, as on phylogenetic tree of HA genes (fig.3).

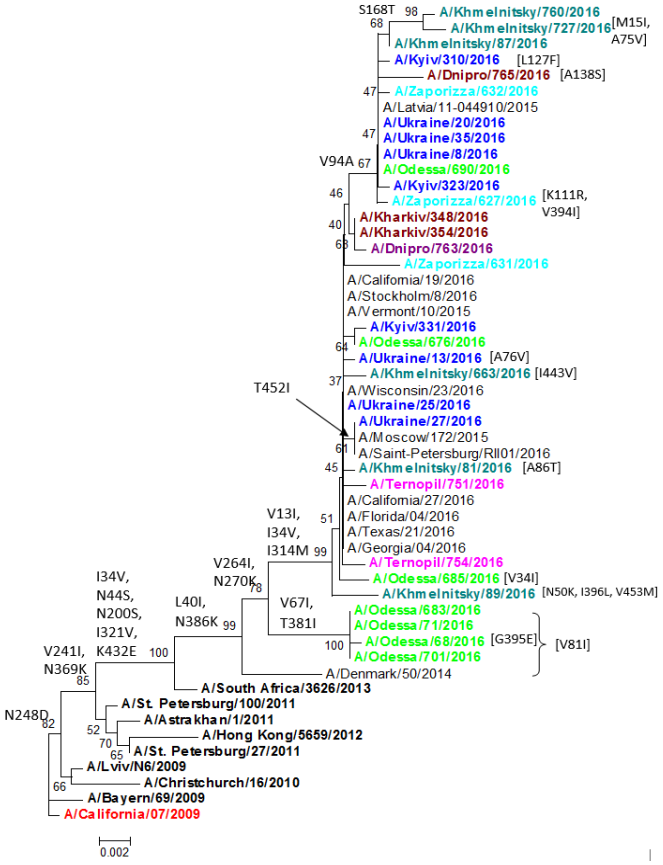

Fig.3. Phylogenetic tree of the NA gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

All discovered isolates had amino acid substitutions – I34V, N44S, N200S, I321V, K432E, except reference strains. Most of Ukrainian isolates belonged to group 6B.1 and acquired substitutions – V13I, I34V, V264I, N270K, I314M. Part of these viruses had mutation V94A.

Group 6B.2 included isolates from Odessa, viruses acquired substitutions V67I (valine to isoleucine), T381I (threonine to isoleucine). Isolate A/Odessa/68/2016 had unique acid substitution G395E.

All discovered viruses retain susceptibility to oseltamivir and zanamivir.

Comparison of non-structure protein (NS1) genes.

Viral NS1 protein plays a central role in counteracting host cell processes that try to interfere with viral replication.

In 2015-2016 epidemic season in Ukrainian isolates amino acid substitutions D2E, N48S, and E125D were identified in the NS1 protein. These mutations were absent in isolates in 2014-2015 epidemic season. Substitutions D2E and E125D occurred in 70% Ukrainian viruses and N48S in 12,5% of sequenced viruses.

Ukrainian isolates 2015-2016 season have been divided into two groups. The second group included 6 isolates from Odessa and 1 isolate from Dnepropetrovsk. In these group substitutions D2E, N48S, and E125D were absent, but isolates had unique point substitutions – I18V, V129I, I182V (fig.4).

An EpiFlu database search revealed that the frequency of substitutions D2E and E125D in NS1 protein of influenza A(H1N1)pdm09 viruses drastically increased in less than 1 year from 10% in 2015 in the Southern Hemisphere epidemic season to 74% in 2015/2016 in the Northern Hemisphere epidemic season [8].

Conclusions. Genetic analysis of influenza A(H1N1)pdm09 viruses circulating in Ukraine in the 2015/2016 epidemic season showed that all of them were similar to the vaccine strain recommended by WHO. Viruses had acquired amino acid substitutions in HA molecule antigenic sites, which can lead to antigenic changes at the next epidemic seasons. Although new genetic subgroups have emerged in 2015-2016 epidemic season, the A(H1N1)pdm09 viruses received were antigenically similar to the vaccine virus A/California/7/2009 and retain susceptibility to oseltamivir and zanamivir

Detailed analysis of substitutions in the protein encoded by internal gene NS1 showed that most of Ukrainian viruses acquired specific amino acid changes: D2E, N48S and E125D. E125D in NS1 is known to be one of the key substitutions involved in shutdown of host mRNA transport, restoring inherent disability of A(H1N1)pdm09 virus to efficiently control human cell gene expression. NS1 of all seasonal human influenza viruses (H1N1 seasonal and H3N2) contains D125 that interacts with cellular cleavage and

polyadenylation factor 30 (CPSF30)6. Interaction with CPSF30 is absent in most animal-adapted strains, so E125D substitution can be considered a milestone in host adaptation of influenza A(H1N1)pdm09 virus.

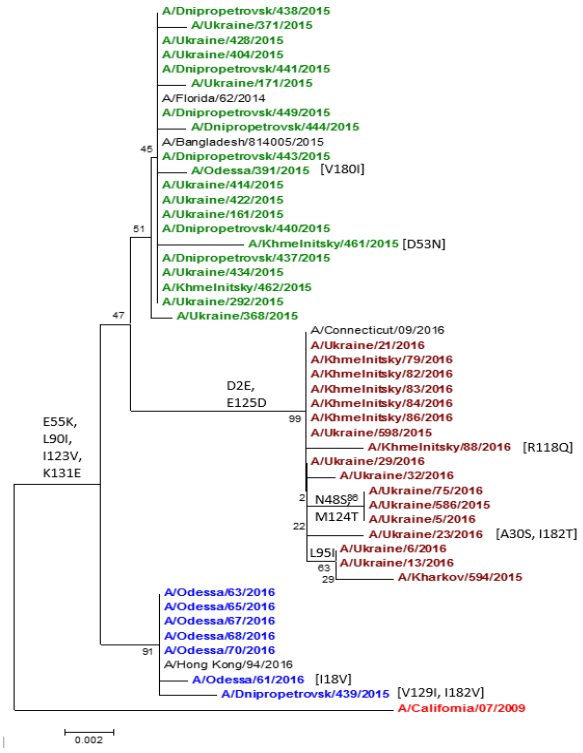

Fig.4. Phylogenetic analysis of the NS1 gene of influenza A(H1N1)pdm09 viruses isolated during 2015-2016 epidemic season

The observed rapid spread of influenza A(H1N1)pdm09 viruses with no significant antigenic changes in HA can be speculatively explained by increased transmissibility, as well as by increased virulence or by combination of both. The possible link between transmissibility or virulence and described changes in NS1 internal gene in influenza A(H1N1) pdm09 viruses awaits experimental proof [8].

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МОЛЕКУЛЯРНО-ГЕНЕТИЧНІ ОСОБЛИВОСТІ ПОВЕРХНЕВИХ ТА НЕСТРУКТУРНИХ БІЛКІВ ПАНДЕМІЧНИХ ВІРУСІВ ГРИПУ A(H1N1)PDM09 В СЕЗОНІ 2015-2016 РОКІВ

Метою дослідження було виявити молекулярно-генетичні зміни в генах гемаглютиніну (HA), нейрамінідази (NA) та неструктурного білку (NS1) пандемічних вірусів грипу, що циркулювали в Україні в 2015-2016 роках. Зразки були проаналізовані методом полімеразної ланцюгової реакції (ПЛР) в реальному часі. Філогенетичні дерева будували в програмі MEGA 7. 3D структури будували в програмі Chimera 1.11.2rc. Віруси, виділені в Україні в сезоні 2015-2016 років, належать до генетичної групи 6B, в якій в цьому сезоні виникли дві нові підгрупи 6B.1 та 6B.2, за генами HA та NA. Ці підгрупи визначаються специфічними для них амінокислотними заміщеннями. В білку NS1 були виявлені нові амінокислотні заміщення D2E, N48S та E125D в сезоні 2015-2016 років. В антигенних сайтах HA були виявлені специфічні заміни, проте віруси зберегли подібність до вакцинного штаму. Білок NS1 набув заміщення, пов'язане з підвищенням вірулентності вірусу грипу.

Ключові слова: віруси грипу A(H1N1)pdm09, амінокислотне заміщення, антигенний сайт, не структурний білок.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ ПОВЕРХНОСТНЫХ И НЕСТРУКТУРНЫХ БЕЛКОВ ПАНДЕМИЧЕСКИХ ВИРУСОВ ГРИППА A(H1N1)PDM09 В СЕЗОНЕ 2015-2016 ГОДОВ

Целью исследования было определение молекулярно-генетических изменений в генах гемаглютинина (HA), нейраминидазы (NA) и неструктурного белка (NS1) пандемических вирусов гриппа, которые циркулировали в Украине в 2015-2016 годах. Образцы были проанализированы методом полимеразной цепной реакции (ПЦР) в реальном времени. Филогенетические деревья построили в программе MEGA 7. 3D структуры построили в программе Chimera 1.11.2rc. Вирусы выделенные в Украине в сезоне 2015-2016 годов, принадлежат к генетической группе 6B, в которой в этом сезоне возникли две новые подгруппы 6B.1 и 6B.2, по генам HA и NA. Эти подгруппы определяются специфическими для них аминокислотными замещениями. В белке NS1 были обнаружены новые аминокислотные замещения D2E, N48S и E125D в сезоне 2015-2016 годов. В антигенных сайтах HA были обнаружены специфические замещения, но вирусы сохранили подобие к вакцинному штаму. Белок NS1 приобрел замещение, связанное с повышением вирулентности вируса гриппа.

Ключевые слова: вирусы гриппа A(H1N1)pdm09, аминокислотное замещение, антигенный сайт, неструктрурный белок.

UDC 578.85/.86

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POTYVIRUSES INFECTING VEGETABLE CROPS IN UKRAINE

This paper describes detection of some potyvirus infecting vegetable crops in Ukraine. Collected samples were screened for the presence of Zucchini yellow mosaic virus and Watermelon mosaic virus-2. Obtained isolates of Zucchini yellow mosaic virus were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup AI. According to the topology of Neighbor-Joining tree based on sequences of NIb-CP genome region obtained WMV-2 isolates showed that belong to group G1. Viruses infecting cucurbits in Ukraine presented by phylogenetic groups widespread in Europe.

Keywords: viral diseases, Potyvirus, vegetable crops.

Introduction. Watermelon mosaic virus 2 (WMV-2) and Zucchini yellow mosaic virus (ZYMV) belongs to Potyvirus genus, Potyviridae family [1]. In experimental conditions, Watermelon mosaic virus 2 infects more than 170 plant species from 26 families. However, cucurbitaceous plants (Cucurbitaceae family) are the major natural hosts for viruses, which were found in both field and greenhouse conditions. ZYMV infects 15 plant species from 7 different families. An occurrence of ZYMV was reported from more than 50 countries. It causes yield losses ranging from 25 to 50 % depending on the pathogenicity of the virus strain [2].

Vegetable crops are widely cultivated in Ukrainian fields. Through characterization of viral population possible migration patterns of ZYMV and WMV-2 dissemination from other countries to Ukraine as well as from Ukraine to other countries may be determined.

Therefore, current study was aimed at detection and characterization of viruses infecting vegetable crops in Ukraine.

Materials and methods. *Vegetable* plants collected from different regions of Ukraine with virus-like symptoms were the objects of this study. Plant sample collection based on the visual symptoms is considered to be the simplest and most common method. For this study, we collected samples with typical viral symptoms under open ground conditions in Kyiv, Poltava, Zhytomyr, Vinnytsya, Odesa, Mykolaiv and Cherkasy regions of Ukraine during 2013-2015 years.

For detection of virus antigens, we conducted DAS-ELISA with commercial test systems of Loewe (Germany) according to the manufacturer's recommendations in 96-well polystyrene plates (Labsystem, Finland). For ELISA, plant samples (vegetative organs and fruits) were homogenized in 0,1 M PBS + 0,001 M EDTA (1:2, v/v) with following sedimentation at 4000 rpm for 20 min at 4^0C using PC-6 centrifuge [3]. Such homogenate was used for ELISA. Optical density values were registered using ELISA reader Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at the wavelength of 405/630 nm [4]. Total RNA was extracted from plant samples using RNeasy Plant Mini kit (Qiagen, UK). RT-PCR was accomplished using specific primers to NIb-CP region of WMV-2 and ZYMV (expected product size – 800 bp, 600 bp respectively) [5]. This genome region is variable among different subgroups, and used for determination of group attribution of ZYMV and WMV-2 [2, 6,7].

Then obtained amplicons were purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big