Table 2. **Sequence changes in Ukrainian isolates of PSTVd**

Item #	Isolate name	Sequence Changes *	Identity	Symptom severity (tomato)
1	Odessa-07/73B	A120C, –A121, C256A, A310U, +U313a	VNIIKH-07/42M (EU879921) Russia	Mild
2	Sokol'skii-08/80B_09	A120U, –A121 A310C, +U313a	PSTVd.021 (X76844) Poland	Mild
3	Sokol'skii-08/80B_10	A120U, –A121 -A126, A310U, +U313a	Unique	Mild
4	Obrit-08/82B	A120C, –A121	PSTVd.125 (Bugry-97) Russia	Intermediate
5	Priekul'ski-08/86B	A120C, -A121	PSTVd.125 (Bugry-97)	Intermediate
6	Panda-08/87B_09	A120U, –A121, C256U, +U101a, A310U, +U313a	Unique	?
7	Panda-08/87B_10	A120U, –A121, C256A, A310U, +U313a	Unique	Mild
8	Rodich-08/88B	A120C, –A121	PSTVd.125 (Bugry-97)	Intermediate

Compared to PSTVd-Intermediate strain (V01465).

Discussion. Russian isolates of PSTVd appear to form two groups/populations: one population can be considered to be derivatives of Russian isolate Bugry-97 (PSTVd.123 = EF044304); a second population contains derivatives of Russian isolate Onega-Premier-94 (PSTVd.125 = EF044303). Both populations differ from the type strain (PSTVd.018 = V01465) by i) the absence of A121 and ii) the presence of a single A120U or A120C substitution. All eight Ukrainian isolates sequenced in this study are members of these two populations. Five isolates belong to the PSTVd.125 population containing an A120C substitution found exclusively in Russian isolates. Isolates Panda-09/87B_09 and its variant Panda-09/87B_10 from Chernigov also contain a second substitution (i.e., C256U/A) that is present only in Russian isolates. Symptoms induced by PSTVd.123 and 125 on Rutgers tomato were very similar to those of the type strain. Several other Ukrainian isolates, in contrast, induced only mild symptoms. Thus, Ukrainian isolates of PSTVd resemble Russian isolates in both nucleotide sequence and biological properties.

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ETIOLOGICAL PROGNOSTICATION OF INFLUENZA EPIDEMICS IN UKRAINE

Етіологічне прогнозування спалахів грипу використовується для передбачення епідемій, розрахунку очікуваних рівніе захворюваності та смертності, а також для вибору стратегії профілактики. Оскільки на Україні вакцини не виробляються, особливу увагу слід приділяти ідентифікації основних інфекційних агентів. В статті наведено аналіз етіологічних прогнозів епідемій грипу в Україні за останні 12 років.

An etiological prognostication influenza outbreak is used for epidemic forecast, calculating expected morbidity and mortality and choosing preventive strategies. As influenza vaccines aren't produced in Ukraine, attention should be paid on identification leading infectious agent. It was analyzed etiological prognoses of influenza epidemics in Ukraine through last 12 years.

Influenza is a highly contagious acute viral infection of the respiratory tract that can affect nasal and mucous, bronchi and even alveoli. This infection might present an endemic, epidemic or pandemic behavior [4]. Etiological prognostication influenza outbreaks is used for epidemic forecast, calculating expected morbidity and mortality, choosing the most effective preventive strategy, planning of controlling and prophylactic measures.

Prognostication influenza epidemics requires for many conditions and only comprehensive derivative information as the final link of annual epidemiological surveillance in Ukraine. Annual outbreaks in different countries are provoked by high variability and transmission influenza viruses, especially for North hemisphere region [2]. It should be noted, periodic shift of influenza viruses cause pandemics with the high morbidity and mortality level. New pandemic influenza A(H1N1) virus, that emerged in the April 2009, have been differed significantly from previous epidemics. This fact has given rise to new problems and tasks [5].

Composition of etiological prognosis is important for predicting annual epidemics. More over, immunity against influenza viruses is strain specific. This means, that only relevant vaccines should be used for influenza prophylactic. An influenza vaccine does not produce in Ukraine. In this reason, we should pay more attention to leading infectious agent of next season for vaccines purchase.

The main aim of our work was to analyze the etiological prognostication of influenza epidemics in Ukraine during last 12 years.

Materials and methods. Materials: nasopharyngeal swabs and autopsy materials from patients, received from the different areas of Ukraine, field isolates of influenza viruses 1998-2010 seasons, strain specific serum for HIA identification.

Methods: hemagglutinin inhibition assay with using chicken and guinea pig red blood cells; real-time RT-PCR analyses with using CDC primers and adopted protocols [1].

Results and discussion. We determined that for relevant etiological prognostication influenza outbreaks at strain level is necessary to obtain the information concerning:

1) The etiological structure of influenza virus populations during previous 5 years in our country;

2) Influenza and ILI (influenza-like illnesses) morbidity level for previous years;

3) Dominated influenza viruses in the world during previous 5 years;

4) New viruses emerged in the world;

5) Intensity of epidemic process in the world for previous years.

Our prognoses are based on statistically-calculated values with including fundamental epidemiology basis and experience of previous pandemics which defined by WHO experts.

We analyzed influenza epidemic situation in Ukraine during previous 12 years (systematic virological surveillance) and have found the clear epidemiological and virological regularity of this disease, which can be a very reliable basis for predicting future epidemics.

During 1998-1999 epidemic season the new virus A/Sidney/5/97 (H3N2) was predicted as a leading infectious agent of epidemic in Ukraine, that was entirely approved. That virus strain was isolated in Volynska and Cherkasska regions. More over, another virus strain (A/Yohannezburh/33/94 (H3N2)) was isolated in Odessa. The diversity among influenza virus populations in Odessa can be explained by the special geographical location of this city and presence of large seaport. These circumstances increase the ability of coming agent from other areas.

At 1999-2000 epidemic season influenza virus type B was prognosed as major infectious agent in Ukraine. However, most isolated viruses belonged to A/Panama/2007/99(H3N2) strain, which were isolated in Kiev and Kiev region., Like previous season difference between populations of influenza viruses observed in Odessa. There have been isolated A(H1N1)/Bern/7/95-like influenza viruses. Thus, the etiological prognosis wasn't confirmed for this influenza season.

Influenza virus type B also was expected as a dominant agent in the next 2000-2001 epidemic season in Ukraine. However, the prognosis wasn't approved again. The leading infectious agent of that outbreake was A(H1N1)/New Kaledoniya/20/99-like influenza viruses. Moreover, the amount of isolated viruses was considerable during the epidemic – 56 isolates in 9 regions. It has provided representativeness of virological data and more exact completion of etiological prognostication for the next epidemic season.

We prognosed B/Sychuan/379/99 influenza virus as a leading infectious agent during 2001-2002 epidemic season. Prognosis was completely confirmed, despite the low intensity of the epidemic process during that season. Such viruses were isolated in three regions of Ukraine.

Next year B/Sychuan/379/99 strain have been prognosed as a leading infection agent of epidemic again. Perhaps, the level of intensity the influenza outbreak hasn't enabled this strain realize fully in previous season. However, A(H1N1)/New Caledoniya/20/99 strain has been dominated during the epidemic So, prognosis was not confirmed [3].

A/Fudzyan/411/02 (H3N2) strain was predicted as the leading infection agent in 2003-2004 epidemic season. Despite of the low intensity the influenza epidemic, the greatest number of isolated viruses belonged to subtype A(H3N2), which is related to both A/Panama/2007/99 strain and A/Fudzyan/411/02 strain, as prognosed.

During the 2005-2006 epidemic season in Ukraine the new virus A/Californiya/7/2004 (H3N2) was predicted as a leading agent. Circulated viruses were similar to the strain A/Californiya/7/2004. Therefore the etiological prognosis was approved.

The majority (45,9 %) of isolated viruses in 2007-2008 were related to the vaccine virus A/Brisbane/59/07 (H1N1). A small part of influenza viruses (28,8 %) were related to the B/Floryda/4/06 strain of B/Yamahata/16/99 genetic branch.

The higher proportion of isolates during the 2008-2009 outbreak were related to A/Brizben/10/07 (H3N2) strain (83 %). It was only 16 % of type B influenza viruses that were similar to the B/Brisbane/60/08 strain, which belonged to the V/Viktoriya/2/87 genetic branch. And only 2 isolates (1 %) were related to A (H1N1) subtype.

Although, B/Brysben/60/2008 strain had been predicted as the main infection agent, the leading agent of 2009-2010 pandemic season were A/California/07/2009-like viruses.

Referencing of predicted and actual infection agent of influenza outbreaks in Ukraine are shown in Table 1.

Table 1. **Conformability between predicted and actual infectious agent of influenza epidemic in Ukraine for 12 epidemic seasons**

Season	Predicted agent	Actual leading agent	Correspondence
1998-1999	A/Sydney/7/95 (H3N2)	A/Sydney/7/95 (H3N2)	Yes
1999-2000	B	A/Panama/2007/99 (H3N2)	No
2000-2001	B	A/New Caledonia/20/99 (H1N1)	No
2001-2002	B/Sichuan/379/99	B/Sichuan/379/99	Yes
2002-2003	B	A/New Caledonia/20/99 (H1N1)	No
2003-2004	A/Fudzyan/411/02 (H3N2)	A/ Panama/2007/99 – like (H3N2)	serotype – yes
2004-2005	A/Fudzyan/411/02 (H3N2) B/Shanghai/361/02	B/Dzhanhsu/10/03 (B/Shanghai/361/02-like)	Partly – yes
2005-2006	A/California/7/04 (H3N2)	A/California/7/04 (H3N2)	Yes
2006-2007	A/Wisconsin/67/05	A/Wisconsin/67/05	Yes
2007-2008	A/Brisbane/59/07 (H1N1)	A/Brisbane/59/07 (H1N1)	Yes
2008-2009	A/Brisbane/10/07 (H3N2)	A/Brisbane/10/07 (H3N2)	Yes
2009-2010	B/Brisbane/60/08 A/California/07/09	A/California/07/09	Partly – yes

Conclusions. We have concluded etiological prognoses of influenza epidemics in Ukraine through last 12 years. Data toward accordance of prognoses and actually leading strain of influenza viruses in country has shown that prognoses had been confirmed entirely in 50%. In 25% of cases prognoses were confirmed partly. And in 25 % of cases prognoses were not justified.

The demand of annual etiological prognostication is determined by the strain-dependent immune response to influenza viruses. It means that only actual vaccines must be used for the prophylactic of flu. An influenza vaccine does not produce in Ukraine. In this reason, we should pay more attention to leading infectious agent of next season for vaccines purchase.

Thereby, 12 epidemics have happened during surveillance influenza viruses. Our etiological prognoses were completely or partly approved in 75 % of cases.

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THE CHERRY LEAF ROLL VIRUS (CLRV) ON WALNUT IN THE REPUBLIC OF MOLDOVA

Проведено тестування бруньок 6 сортів волоського горіху, відібраних на комерційних плантаціях, на наявність вірусу скручування листя черешні (родина Secoviridaea, рід Nepovirus). Для детекції CLRV у зразках використовували специфічну антисироватку при проведенні ІФА, в модифікації DAS, та ISEM. Загальна кількість протестованих зразків становила 315. Вірус був ідентифікований у 14 зразках рослин сорту Когалнічану. Проведено механічне зараження рослин Nicotiana occidentalis 37B, потім вірус перенесено на рослини Chenopodium quinoa Willd, які використовували для отримання очищених препаратів. Була отримана поліклональна антисироватка до CLRV з титром 1:4000.

Bud samples from 6 varieties of walnut from commercial plantation were collected and tested for the presence of cherry leafroll virus (family of Secoviridae, genus of Nepovirus). The specific antisera was used for detection CLRV in samples by DAS-ELISA as well as by ISEM. There were tested 315 samples in all. The virus was found in 14 samples of var. Kogalnichanu. The virus was transmited mechanicaly on the tobacco plants Nicotiana occidentalis 37B and then passed on Chenopodium quinoa Willd that was used for obtaining the purified preparation. There was obtained the policlonal antiserum to the selected isolate CLRV with the titre 1:4000.

Introduction. The Republic of Moldova has a favorable natural conditions and ancient traditions to promote a culture of walnut. Early last century, Moldova exported 10 000 tonnes of fruit and nut plant material to neighboring countries, grown exclusively by seed. Lack of theoretical and practical skills of vegetative propagation has led to irretrievable loss of many valuable forms of culture. Since 60-ies 19th century in Moldova was selected varieties according to certain criteria. So, in 1980 were legitimized five varieties, and in 2009 there were already assortment of 14 items. At the same time, scientists of Moldova are developing technology of vegetative propagation of walnut, which makes it possible to produce annually up to 250,000 grafted plants per year. Production of such a large number of planting material without proper fitosanitary control is a threat of widespread destructive virus in this culture of cherry leaf roll virus (CLRV).

This virus was first described in 1976 in Italy (Savino et al., 1976). Subsequent studies have identified three isolates of the virus causing yellow mosaic disease, ring spot and black line on union zone. The first two of the disease got its name based on the symptoms they cause on the herbaceous indicators. Isolate black line is associated with the incompatibility of sorts with some rootstock and is the cause of mass mortality of plants in the traditional walnut-growing this crop (Mirchetich et al., 1982; Cooper, 1980; Quacquarelli and Savino, 1977; Nemeth et al., 1982; Delbos et al., 1983; Kolber et al., 1983). Established that CLRV transmitted vegetatively and pollen. According to Kolber and Nemeth (Kolber and Nemeth, 1983) the percentage of infected seeds sampled from diseased trees may reach 92.8%. Same number of infected seedlings grown from seeds of infected trees, reaching more than 4% (Quacquarelli and Savino, 1977). As regards the transfer CLRV pollen, found that during the pollination of about 19% of the trees can be infected with the virus under investigation. Based on the above and taking into consideration the annual growth of walnut plants produced in the country, we have initiated a study on the infected trees in mother plantation.

Materials and methods. The material of study was axillary buds annual shoots of mother walnut trees. Diagnosis CLRV was performed by ELISA (Clark and Adams, 1977) using commercial diagnostic kits firms "LOEWE" and immunosorbent electron microscopy (ISEM). Primary mechanical transfer of virus to herbaceous indicator Nicotiana occidentalis 37B was performed by rubbing leaves an indicator of buds extracts in phosphate buffer, pH 7.4. The same buffer is used to transfer CLRV from infected tobacco plants to Chenopodium quinoa Willd. to obtain of purified virus particles. Purification of the virus was carried out using standard stages of homogenization, clarification of leaf extract, the concentration of particles by high-speed centrifugation and fractionation in sucrose density gradient. To obtain the antiserum short schema of immunization is used with subsequent double reimmunization experimental animals at 60 and 70 from the beginning of the cycle of immunization. Antibody activity was determined by ISEM with the use of extracts from systemically infected leaves indicator Ch.quinoa.

Resalts and discussion. The study of virological situation in the plantations of walnut were started by us with visual inspection of high-quality mother plantation at specialized commercial nursery "AMG KERNEL" for the production of walnut trees. From each tree, regardless of the presence or absence of their symptoms, we selected