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# PLENARY SESSION

# THE ORIGINS AND EVOLUTION OF THE VIRUS WORLD

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Viruses and other genetic parasites are associated with virtually all forms of prokaryotic and eukaryotic cellular life. Furthermore, these selfish agents dominate the biosphere, both numerically and in terms of genetic diversity. Phylogenomic analysis of the vast and rapidly growing collection of viral genomes has led to a concept of Virus World. This concept recognizes that selfish elements do not have a single common ancestry, but form a network, nodes of which are connected by viral hallmark genes, those that encode key proteins involved in replication of viral genomes and virion morphogenesis. The hallmark genes are shared by diverse genomes of viruses and selfish elements and have only extremely distant homologs in cellular life forms, except for cases of apparent acquisition from viruses. This pool of ancient, essential genes defines the Virus World that appears to have been continuous since the earliest, pre-cellular stages in the evolution of life that are still understood only in general terms.

In contrast, recent analyses of viruses and capsid-less selfish elements of eukaryotes resulted in a coherent evolutionary scenario offering a small number of ancestors among bacterial viruses and selfish elements. In particular, origins of retroviruses including HIV were traced to eukaryotic LTR retrotransposons that, in turn, likely originated from the bacterial group II introns. The most recent events in retrovirus evolution are epitomized by cross-species transmission of SIV from chimpanzee to humans, followed by a burst-like diversification and global dissemination of HIV-1. On a larger scale, the most recent gene and genome network analyses of the dsDNA viruses by Eugene Koonin group revealed robust hierarchical modularity with some modules combining diverse viruses infecting bacteria, archaea and eukaryotes. These analyses provided statistical basis for the formal identification of the hallmark genes thus supporting the Virus World concept that was first advanced 10 years ago.

# PROBLEM ISSUES RELATED WITH THE EMERGENCE INFLUENZA VIRUSES AND PROSPECTS FOR VACCINATION

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The relevance of emergent viral infections, including influenza, increases every year. High evolutionary potential of influenza viruses, a wide range of natural hosts cause a large variety of serotypes. Always there is a risk of adaptation of new reassortant viruses in existence in the human population, there is a risk of formation of new parasitic systems [1]. It requires constant analysis of risk (pathogenicity, the ability for pandemic spread, particularly immune response, etc.), related to novel viruses that can cause human disease.

At present, special attention should be paid the following human pathogenic influenza viruses that have emerged in recent years: a pandemic influenza virus A(H1N1) pdm09 (2009), avian influenza viruses A(H5N1) (1997), A(H9N2) (1998), A(H7N7) (2003), A(H7N3) (2004), A(H7N9), A(H10N8) (2013), A(H5N6) (2015).

VirusA(H1N1)pdm09, causing the epidemic in 2009-2010., later was attributed to seasonal influenza viruses. However, studies have shown [8], in fatal cases, etiologically related virus, low levels of CD206+ cells (marker of alternatively activated macrophages marker in lung) were found when compared with seasonal influenza virus ($P<0.05$), and the ratio of CD206/CD14+ cells was 2.5-fold higher in seasonal and noninfluenza group compared with influenza A (H1N1)pdm09 ($P<0.05$). The authors suggest the use of CD206+ cells number for differentiation between influenza A (H1N1)pdm09and seasonal influenza virus in lung tissue of fatal cases. In addition, the rise of the epidemic of influenza in Ukraine in the season 2015-2016, caused by the virus, has shown a tendency to increase its pathogenic potential against the background of the conservation of antigenic properties. The number of cases of influenza and acute respiratory viral infections has exceeded 4 million, and the number of deaths on 02.25.2016 amounted to 338. In this flu season in Ukraine were vaccinated only about 130 thousand persons (0.3% of the population), which could not affect the intensity of the epidemic process.

During 2003 - 2016 (on 06.13.2016) in 16 countries registered 851 cases among people, including 450 deaths (52.9%) caused by the highly pathogenic avian influenza virus A (H5N1) [10]. The increased incidence (145 cases) and reduced mortality (29.0%) in 2015 compared with previous years can be linked, on the one hand, the expansion of surveillance and detection of light clinical forms, on the other hand, we cannot exclude the existing risk of the viral adaptation to human population. This last assumption requires careful study.

Influenza A(H9N2)also is capable of causing disease in humans. It can still be gene donor for other avian influenza viruses, including pathogenic viruses for humans such as A(H7N9) andA(H10N8) [9]. The first cases of flu among the people associated with the virus A (H7N9) (triple reassortant avian viruses), were registered in China in 2013. On 16.02.2016 itreported 729 laboratory-confirmed cases, including 282 deaths (38,7%) [3].According to the assessment of the international group of experts this virus is estimated as dangerous, and this situation needs study and monitoring. There are reports of sporadic probable cases of virus transmission from person to person [2]. There are currently data on 15 cases aware of 15 cases of influenza in humans caused by a new virusA(H5N6)including at least four deaths (on 26.07.2016).

Cases of human infection with influenza virusA (H10N8) registered in China in 2013 and 2014 (3 cases, including 2 deaths)[7]. Influenza viruses A (H7N3), A (H7N7) are highly pathogenic for birds. These viruses can cause flu-like symptoms with conjunctivitissn humans, which are observed during poultry outbreaks of this infection [4, 5, 6].

Thus, influenza viruses of people, birds and mammals need constant virological monitoring at both the national level and on a global scale. Such monitoring also provides the selection of candidate strains for production of highly effective vaccines in case one or another virus acquires the ability to a wide transmission from person to person.


1. Фролов А.Ф., Задорожная В.И. Молекулярная эпидемио-логия вирусных и прионныхболезней. – Киев: ДИА, 2010. – 280 с.
2. Fang Ch.-F., MaM.-J., ZhanB.-D.et al Nosocomial transmission of avian influenza A (H7N9) virus in China: epidemiological investigation // BMJ. – 2015. - 351:h5765: http://dx.doi.org/10.1136/bmj.h5765.
3. H7N9 situation update. – Food and Agriculture Organization of the United Nations. – 1 March - 2016: http://www.fao.org/AG/AGAInfo/programmes/en/empres/H7N9/situation_ update.html.

4. Hirst M., Astell C. R., Griffith M. et al. Novel avian influenza H7N3 strain outbreak, British Columbia // Emerg. Infec. Dis. – 2004. – 10 (12). – P. 2192-2195.
5. Koopmans M., Wilbrink B., Conyn M. et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands // Lancet. – 2004. – 363 (9409). – P. 587-593.
6. Lopez-Martinez I., Balish A., Barrera-Badillo G. etal. Highly pathogenic avian influenza A(H7N3) virus in poultry workers, Mexico, 2012 // Emerg.Infect.Dis. –. 2013. – 19 (9). – P. 1531-1534.
7. Qi W., Zhou X., Shi W. et al. Genesis of the novel human-infecting influenza A(H10N8) virus and potential genetic diversity of the virus in poultry, China // Euro Surveill. – 2014. - 19 (25). - http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20841.
8. Rodriguez-Ramirez H.G., Salinas-Carmona M.C., Barboza-Quintana O. et al.CD206+ cell number differentiates influenza A (H1N1)pdm09 from seasonal influenza A virus in fatal // Mediators of Inflammation/ - 2014. – V. 2014. - http://dx.doi.org/10.1155/2014/921054
9. Sun Y., Liu J.H9N2 influenza virus in China: a cause of concern // ProteinCell. – 2015. – 6(1). – P. 18-25.
10. WHO: Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2016. - http://www.who.int/influenza/human_animal_interface/2016_06_13_tableH5N1. pdf?ua=1

## OVERVIEW AND LATEST UPDATES ON ZIKA VIRUS

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The 1st of February 1st of this year, the World Health Organization (WHO) declared Zika virus to be a Public Health Emergency of International Concern (PHEIC) following reports of microcephaly in newborns or Guillain-Barrй syndrome in adults appearing concomitantly with Zika virus outbreaks. Zika virus is an arbovirus transmitted by

mosquitos. Zika virus had previously been considered to cause only a mild disease, with symptoms such as fever, rash, arthralgia, muscle pain and conjunctivitis. Before 2007, when an outbreak of the virus affected 70% of the people in the Micronesian Yap State, Zika virus had only been seen in Africa and South-East Asia. Six years later, a Zika virus epidemic occurred in French Polynesia infecting more than 32 000 people. The virus was probably introduced to the Americas two years ago, and the first report of autochthonous transmission in Brazil was as late as May 2015. Zika virus has now spread to 31 countries and territories in the Americas, including Puerto Rico and the USA. An update on the Zika virus epidemic in the Americas and its possible causal relation to microcephaly and Guillain-Barrй will be discussed.

## ECOLOGICAL AND EPIDEMIOLOGICAL FEATURES OF WEST NILE FEVER AT THE MODERN STAGE

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**Background**. Particular concern and interest of multidisciplinary specialists at the present stage cause infections that belong to the group of natural focal zoonotic especially dangerous infections (EDI). These pathogens have high epidemic potential and make huge biological threat, therefore, by recommendations of WHO experts, some of them have been referred to the list of diseases that are controlled at international level.

One of the most actual natural focal infections is West Nile fever (WNF), which belongs to the group of transmissible zooantroponotic arboviral diseases transmitted by mosquitoes [1, 2].

WNF agent firstly was isolated from the blood of patients with fever in 1937 in Africa, West Nile district of Uganda that was given the name of this pathogen. Virus of WNF belongs to the genus *Flavivirus* family *Flaviviridae*. WNF virion consists of protein C and lipoprotein membrane, which houses two glycoproteins M and E that are the structural proteins and nonstructural protein NS1-NS5. RNA contains one extended

ORF, flanked on both sides by end 5'- and 3'- UTR, which play an important regulatory function in RNA replication and translation. RNA translation initiation occurs by cap-including mechanism [3].

Due to the phylogenetic properties of the WNF virus, it is distinguished its multiple genotypes, some of them contain subgroups, that cause uneven spatial distribution and severity of clinical manifestation of the disease. Genotype 1 contains three subgroups 1a, 1b, 1c. Strains of this genotype is circulating mainly in Europe, Africa, North America, Australia, and the disease is characterized by severe clinical forms in human, birds and horses. Virus strains of the genotype 2 were first identified in Africa and Madagascar, cause light or even asymptomatic clinical forms of the disease. Also there are distinguished genotypes 3 and 4, ones strains were isolated in the Czech Republic and the Caucasus respectively, clinical and epidemiological features of which have not been insufficiently studied [3].

Nowadays WNF agent is characterized by planetary distribution; it is registered at all continents except Antarctica. It is considered that primary foci of WNF occurred in the African and Eurasian continents. The biggest outbreaks have been described in Israel (1956) [6], the western Mediterranean, in Russia (1962-1964 and 1999), in Belarus (1970-1980). Then in Romania outbreak had place (1996-1997), there were serologically verified more than 300 patients and mortality was about 10 %, also in the Czech Republic (1997) and Italy (1998). Large-scale outbreaks of WNF at the American continents emerged in 1999-2001, since cases of the disease are recorded annually [4, 5].

**Aim.** To estimate activity of WNF epidemic process in Ukraine and study about the basic components in formation of stable foci of WNF.

**Methods.** Complex epidemiological method; ELISA serological method ("VectorNile-IgM, "Vector-Best", Novosibirsk, Russia) to research the presence of IgM and IgG to WNF in paired blood sera of 103 patients with unknown seasonal febrile states.

**Results.** First case of WNF in human in Ukraine was recorded in 2006. For today, scientists confirmed the existence of WNF natural foci with virus circulation in the North-West Black Sea and also 12 regions of south-eastern and western regions of Ukraine are considered as enzootic areas [1, 7].

The risk territory of WNF is determined by the availability of adequate circumstances that lead to functioning sustainable WNF foci. The defining parameters in the foci formation are the sum of effective temperatures that ensure full development cycle of mosquitoes as vectors and reservoirs of the virus; water objects on the territory and other ecological and floristic conditions necessary for vectors breeding. Equally important parameter is the faunistic component – a wide range of animals

and birds as reservoirs of infectious agents, identifies areas of the pathogen spreading. It should be noted that in the case of entry of the pathogen in a particular area, migratory wild birds of bog-water complex provide intercontinental virus introduction. Synanthropic and semi-synanthropic birds ensure the circulation of the pathogen in a particular area. Confirmation of this hypothesis is the entry of the virus in the United States in 1999, where previously the agent never circulated, and the rapid spread it throughout the American continents, causing severe cases and deaths among people [5]. Thus, the infection spread rapidly to various continents away from its primary foci.

We have conducted a retrospective analysis of WNF incidence in three sites of one of the western regions of Ukraine based at data of hospital surveillance for patients with seasonal febrile states that did not exclude the diagnosis of WNF. Having regard to the vertical zoning, it should be noted that one of the sites was in the flat landscape area, and two another – in the highlands. It was established that the incidence in three sites averaged 43.2 per 100 thousand populations. Incidence rates were significantly higher in the two sites that were in mountainous areas – 85.2 and 44.9 per 100 thousand populations, while in the area, located in the flat landscape – only 25.9 per 100 thousand populations.

According to our data, the level of protection of the total population against virus infestation is low in the three sites. These results explain the high incidence among this population, where the clinical course had a different character in severity, often – with asymptomatic or flu-like manifestation of the disease.

The territory of Ukraine has all conditions for the formation and function of sustainable WNF foci. Global climate warming observed in recent years, changes in hydrological parameters, favorable flora-fauna features, intensifying the movement of people (air transport, etc.) and other prerequisites stimulate the formation of new foci and expanding borders of the known territories of natural focal EDI, including WNF, also at unexplored regions.

**Conclusions.** Due to the facts that at the present entomological and ornithological research are not conducted, it is unable to fully assess the activity of WNF epidemic process at the whole territory of our country. This explains the need for implementation of adequate control and continuous monitoring of the entire territory of Ukraine on biological threats [7].

1. Vynograd N. Arboviral infections of Northwestern Black Sea / N. Vynograd, O. Yurchenko, D. Dubina // Infectious disease. – 2013. – № 3. – P. 5-9.

2. WHO. International Health Regulation 2005. http://www.who.int/en.
3. Guide to virology: Viruses and virus human and animal infection / Ed. D.K. Lvov. – M.: OOO "Publishing" Medical information Agency", 2013. – P. 228-235.
4. Arnold C. West Nile virus bites back / C. Arnold // Lancet. – 2012. – № 11(12). – P. 1023–1024.
5. Calistri P. Epidemiology of West Nile in Europe and the Mediterranean Basin / P. Calistri, A. Giovannini, Z. Hubalek [et al] // The Open Virology Journal. – 2010. – Vol. 4. – P. 29-37.
6. Marberg K. The natural history of West Nile Fever. Clinical observations during an epidemic in Israel / K. Marberg, N. Goldblum, W. Jasinska-Klingberg // Am. J. Hyg. – 1956. – №64 (3). – P. 259-269.
7. Vynograd Nataliya. Natural foci diseases as a stable biological threat / N. Vynograd // Archivum Immunologiae et Therapiae Experimentalis. – 2014. – Vol. 62 № 6. – P. 445-447. Access mode: DOI 10.1007/s00005-014 - 0316-8.

## USE OF PHYLOGENETIC ANALYSIS FOR STUDYING ORIGINS OF VIRUSES

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All life forms on Earth, both living and extinct, have common origins and their evolutionary history may be represented by a sequence of divergences from common ancestors. The later two life forms diverged from a common ancestor, the more they related to each other. As a science, molecular evolution includes two directions of research. These are aimed at elucidating regularities of alterations of hereditary information in living systems (including precellular and cellular life forms), studying history of life development on Earth, and establishing relationships among the life forms and their genetic material – phylogeny of the forms of life.

The first concept assumes studying frequency and other characteristics of evolutionary changes in macromolecules (nucleic acids and proteins), and also mechanisms and causes governing such changes.

The second direction of research in molecular evolution is focused on reconstruction of evolutionary process of life forms on Earth, establishing relationships among them, and evolutionary classification which is jointly named molecular phylogenetics.

Development of polymerase chain reaction and automated sequencing techniques permitted to obtain nucleotide sequences of plant viruses and analyze their genetic data using computer technologies coupled with approaches of molecular evolution and phylogeny. Molecular evolution is about establishing evolutionary history of a group of homological sequences. Using two plant viruses – Plum pox virus and Tobacco mosaic virus - as examples, here we demonstrate the efficiency of molecular phylogenetics for establishing both strain of the virus (PPV) and time of divergence of a novel isolate/strain (TMV).

## NEW STRATEGIES AGAINST DRUG RESISTANCE TO HERPESVIRUS

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Today there are a large number of virus's pathogens of humans, which affect various tissues of macro organism and have the levels of manifestations from latent to lethal forms. Infectious diseases, those are capable to cause similar clinical diseases at neonates and children by transviral, transplacental or ascending infection, had been united by general abbreviation "TORCH-complex". In last years the noticeable growth of pathology caused by TORCH-group infections (herpes simplex virus of 1 and 2 types (HSV1/2), cytomegalovirus (CMV), Epstein-Barr virus (EBV) et al) are registered. According to the WHO global health survey, in 2012, 140 million and 417 million people between 15 and 49 years of age lived with HSV-1 and HSV-2, respectively. During the past decade, many potent agents have become available against viral infections; however, the increasing clinical use or the abuse of these agents has been associated with the emergence of dangerous drug resistant viral strains. In addition, the dose-limiting toxic effects of the known antiviral agents have been observed

among patients especially immunocompromised individuals. ACV and related nucleoside analogues have been gold standard molecules for the treatment of HSV infections during the past decades. However, the emergence of ACV drug-resistant HSV is rising rapidly with the increasing numbers of transplant and cancer patients. It was feared that resistance might be quick to develop against these drugs through mutations in the viral thymidine kinase and/or DNA polymerase enzymes, registration of which has increased over the last ten years. Viruses use different ways to preserve their genetic information in the body, starting from the integration into the genome of host cells and ending by the formation of episomes. Activation of persistent form of viral infection takes place both under action of external environmental factors and under inhibitory action of other viruses on the immune system. Modern epidemiological studies show that, as a rule, the clinical picture of the infection involves multiple etiological agents. Typically, the range of viral and bacterial pathogens, which are in the same tissues and can interact, simultaneously coexists in macro organism. In our work the changes of the nature of the pathological processes were found, as a result of the interference of the viruses and the differences of the drugs activities against the co-associated viruses in conditions of the mono- and co-infections of cells. Both the increase and inhibition of the drugs activities were detected that may lead to the formation of resistant strains of viruses. The problem of mixed infections requires further laboratory study, the creation of new methods of evaluation and new approaches to therapy. However, many pharmaceutical companies have clearly decided to avoid this area, perhaps governments and granting agencies should do more to support research in this area, as they have done for certain types of vaccine development and defense against bioterrorism.

## A COMPLETE AND LONG-TERM CONTROL OF *PLUM POX VIRUS* IN AREAS WITH ENDEMIC PRESENCE OF THE VIRUS

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The control of *Plum pox virus* (PPV) in areas with endemic presence of the virus was very difficult up to the present time. The first

condition is to use PPV free planting material. The system of certification of planting material is based on the technical and space isolates. PPV free plants of stone fruits in nurseries are derived from mother plants in technical isolate. Certification systems were developed and are recommended by the regional plant protection organizations, e.g. European Plant Protection Organization (EPPO). Growing of PPV free trees in orchards cannot be fully effective in countries and areas where the presence of PPV is endemic. Therefore cultivars resistant to PPV must be used. The problem of resistence to PPV in stone fruits has not been satisfactory solved by traditional breeding until now.

Genetically modified (GM) plum (*Prunus domestica* L.) clone C5 (cv. rHoneySweetr ) was identified as highly resistant to *Plum pox virus* (PPV). It is the first plum cultivar for which it is not possible to transmit PPV through aphid inoculation. The combination of a stone fruit cultivar resistant to aphid-transmitted PPV infection and a rootstock with the same property will result in trees that will remain PPV free for their entire life-time. The rootstock myrobalan PK which is suitable for plum and apricot cultivars cannot be infected with PPV through aphid inoculation, too. The resistance to PPV through aphid inoculation, conditioned by one dominant gene locus, can be used to develop new PPV resistant cultivars with the same level of resistance to aphid-transmitted PPV. Moreover, several plum and apricot cultivars tested through graft inoculation have been reported to be resistant to PPV, but these have not been tested for aphid inoculation.

Hybridizations between PPV resistant rHoneySweetr plum and rDomбcн velkoplodбr or rPozegacar plums with high quality fruits, but very susceptible to PPV, have been carried out. Field trials of plum and apricot cultivars resistant to PPV and grafted on the myrobalan PK rootstock were established to verify the lack of PPV transmission by aphids. The aim of this research is to produce and to make available plum and apricot cultivars grafted on the myrobalan PK that will remain PPV free for the entire lifetime of orchards, providing a long-term stable production for growers, and a dependable supply of healthful fruits for consumers.

# EMERGENCE VIRAL DISEASES OF ANIMALS AND PREDICTION OF BIORISKS

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The abstract deals with some aspects that enhance the ability to predict of biorisks in viral infections, which are referred to as cross-border, such as Crimean-Congo fever, African swine fever, classical swine fever, Gumboro disease, avian influenza, Newcastle disease. We present some data on molecular mechanisms of resistance of animals to these viruses. The attention is focused on an integrated approach during the prediction of certain biorisks at the present stage.

**Keywords:** emergence, infections, viruses, animals, biorisks

Viruses as pathogenic factors of animal diseases, affecting sensitive body are widespread in the world and cause a different course of infection. They often cause significant economic losses and this applies to so-called "economically significant viral infections." Among them diseases such as African and classical swine fever, foot and mouth disease, Newcastle disease, viral encephalitis, influenza of swine and poultry, cattle plague, the plague of small ruminants, cattle bovine spongiform encephalopathy, bluetongue, vesicular stomatitis and others. For example, FMD was informally called "disease that devastates the economy» (economically devastating livestock disease), confirming its great importance for the economy of countries where it occurs.

In this article we considered some aspects that enhance the ability to predict of biorisks in viral infections, which are referred to as cross-border, such as Crimean-Congo fever, African swine fever, classical swine fever, Gumboro disease, avian influenza, Newcastle disease.

Classical swine fever (CSF) is one of the most dangerous infections from an economic point of view, both in wild and domestic population of swine. Currently, the disease is found in more than 60 countries on all continents (except the USA, Canada, Australia, and Scandinavian countries). Countries in Europe, Asia, South and Central America are more affected with this infection, where a pig breeding is well-developed. In recent years, CSF had been thorough studied, a vaccine was

developed and methods for diagnosis were offered, but attempts to completely eliminate the disease have not yet reached a stable success.

More details we considered the question of the epizootic situation of classical swine fever in Ukraine for 10 years period. Also, in parallel we analysed the detection of outbreaks of the disease in the world in 1961-2015 years.

Analyzing the data, one should consider that there is always a threat of an infection spreading as a cross-border disease against a background of international trade relations. In some parts of Europe the CSF virus adopted endemic forms in a population of wild pigs, which creates a constant threat to the population of pigs.

The aim is to explore the epizootic and serological monitoring of CSF pathogen distribution among population of wild and domestic pigs in Ukraine and abroad. Analysis of epizootic data is associated with the data of the wild pig population in different regions of Ukraine.

Revealed positive for CSF wild pig and the analysis of their distribution in regions of Ukraine. According to the analysis of the epizootic situation and calculate the risks of CSF in Ukraine, the country conditionally divided into 4 zones: -zona of slightest risk of CSF; -zone of relative prosperity, -zona of high risk of CSF; -zona trouble -is highest number of outbreaks of CSF.

It was found the changes of pathogen circulation of CSF dynamics in wild fauna in the regions of Ukraine for the period 2001 – 2012 (by Mushtuk I.U. data). Most seropositive animals (from research) found in the Central (14.9%) and the North (12.3%) regions Ukraine, where the virus titers of antibodies to CSF reached to 1: 256. Most CSF virus seropositive animals found in the Central region in Cherkasy (100%) in 2001 and Poltava regions (80%) in 2003. Among the regions of the Northern region of 100% seropositive animals found in the Kiev region in 2002. The smallest number of seropositive wild pigs is marked in Western Ukraine - 5.36%. CSF. Comparative analysis of outbreaks of CSF indicates a trend similarity manifestation of the disease among domestic and wild pigs with two-step intensity periods of epizootic situation in 2000-2004 and 2006-2010.

We reviewed some aspects of other viral diseases, which are of interest to the prediction of risk, namely, Crimean-Congo fever, African swine fever, classical swine fever, Gumboro disease, avian influenza, Newcastle disease.

# POTYVIRUSES INFECTING VEGETABLE CROPS IN UKRAINE

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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.

Symptomatic plant samples were collected in different regions of Ukraine. Collected samples were screened for the presence of *Zucchini yellow mosaic virus* (ZYMV) and *Watermelon mosaic virus-2* (WMV-2). Detection of viral antigens was carried out by DAS-ELISA using commercial test systems. ZYMV caused yellow mosaics, leaf blade deformation, knobs and malformations of fruits. The symptoms of WMV-2 included dark green mosaic, vein banding and dark mottle on leafs, deformation of fruits and stunting.

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). This genome region is variable among different subgroups, and used for determination of group attribution of ZYMV and WMV-2. Then obtained amplicons were purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 (Applied Biosystems, USA). Phylogenetic analysis was conducted using Neighbor-Joining method in MEGA 6.

For ZYMV we obtained following Nib-CP sequences of Ukrainian isolates: ZYMV-10G, ZYMV 5/13 (extracted from *Cucurbita pepo L.* in Poltava region), ZYMV-10P (extracted from *Cucumis melo* in Vinnytsia region), ZYMV-38/14 (extracted from pumpkin (*Cucurbita pepo L)* in Cherkasy region),) i ZYMV-B (extracted from *Cucumis melo* in Cherkasy region). Ukrainian isolates were characterized with high homology (98-100%). Obtained isolates were clustered with isolates from Slovenia, Hungary, Czech Republic, Austria and France within subgroup AI [1]. According to the literature data, subgroup AI includes the most frequently

detected strains from different geographic origin. WMV-2 isolates were also obtained from various plants in different regions: WMV-2G, WMV-21 (extracted from *Cucurbita pepo L.* in Poltava region), WMV-4K (extracted from *Cucumis sativus L.* in AR of Crimea, WMV-3ch and WMV-4ch (extracted from *Cucurbita pepo L.* in Cherkasy region), WMV-63 (extracted from *Cucurbita pepo L.* in Kyiv region). The homology ranged from 94 to 99%. The topology of Neighbor-Joining tree based on sequences of NIb-CP genome region showed that Ukrainian isolates of WMV-2 belong to group G1 [2]. Group G1 consists of non-recombinant isolates reported from different countries [3]. To summarize, viruses infecting cucurbits in Ukraine presented by phylogenetic groups widespread in Europe.

1.Desbiez C, Wipf-Scheibel C., Lecoq H. Biological and serological variability, evolution and molecular epidemiology of Zucchini yellow mosaic virus (ZYMV, Potyvirus) with special reference to the Caribbean islands/ C. Desbiez, C. Wipf-Scheibel, H.Lecoq// Virus Res. .– 2002. – Vol. 85.– P. 5-16.
2.Serological and molecular variability of watermelon mosaic virus (genus*Potyvirus*)/ C. Desbiez [et al/]. // Archives of Virology.– 2007. – Vol. 152, № 4.– P. 201–208.
3.Moradi Z. Diagnosis and molecular variability of Watermelon mosaic virus isolates from North, East, North-East, and North-West regions of Iran / Z. Moradi // Asian Journal of Plant Pathology. – 2011. – Vol. 5, № 3. – P. 115–125.

## VIRUSES AND VIRAL DISEASES OF FISH IN AQUACULTURE

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Fish are very effective converters of feeds into high quality food. The world system of food production, including agriculture, sea fishery, inland fishery, and aquaculture, is based on the ecological aspect, however, we destroy this basis through excessive fish harvest, environmental pollution, and many other ways. By doing this, we threaten the possibility to produce the necessary food products in the future perspective. An important

problem of food safety in Ukraine is food quality, in particular that of fish products. Aquaculture is one of promising branch of animal breeding. The quick growth of this industry is first of all related to drastic decline of wild fish stocks and development of inland water resources. A traditional object of Ukraine's aquaculture is carp. In addition, trout and sturgeon cultures are being actively developed lately. Every year, about 130 thousand tons of trout and 200 thousand tons of carp are grown in Ukraine. Concurrently with the intensification of fish culture processes, new viral diseases appear which inflict significant damage to world aquaculture. More than 250 viruses have been discovered in fish, the majority of which in the countries with highly developed aquaculture (USA, France, Germany, United Kingdom, Italy, Norway, Japan, etc.) [1-3]. However, their incidences in different countries are different. It is known that highly dangerous for aquaculture are diseases caused by RNA-containing viruses, including spring viraemia of carp (SVC), infectious hematopoietic necrosis (IHN), infectious pancreatic necrosis (IHN), and viral hemorrhagic septicemia (VHS), as well as DNA-containing viruses such koi herpes virus (KHV) and sturgeon herpes viruses [4-5].

During monitoring studies carried out in specialized fish farms of Ukraine in 2003-2014, we found infectious pancreatic necrosis virus in some private farms in Chernivtsi, Zakarpattya, Lviv, and Volyn regions as well in the water bodies supplying these farms with water. A virus was isolated when examining rainbow larvae and fingerlings. Phylogenetic studies showed that IPNV isolates circulating on the territory of Ukraine belong to European genotypes and Sp strain.

As for farms specializing in carp rearing, the situation remains challenging. Several studies have been conducted in fish farms located in different regions of Ukraine including Lviv, Rivne, Khmelnitsky, Ternopil, Vinnitsa, Kyiv, Chernihiv, Mykolaiv, Kherson, Donetsk regions. Diagnostics were carried out in a complex taking into account clinical, pathologic, and laboratory studies. Examinations of fish in spring-summer period, in stress conditions, after being transferred from wintering ponds to fattening ones allowed detecting the isolates on spring viraemia of carp virus. It affected carp of different age groups but the clinical signs of viral infection were observed more frequently in age-1 and age-2 fish. Modern virological and molecular-biological methods allowed studying the distribution of spring viraemia of carp in the conditions of freshwater aquaculture. The affection of carp of different age groups with this virus in fish farms of Lviv, Donetsk, Chernihiv, Kyiv, and Odesa regions indicates on its broad distribution in Ukraine. A comparative analysis of G glycoprotein gene sequence of SVCV isolates circulating on the territory of Ukraine, showed that they belonged to Fijan strain and formed the genetic group Ia.

We also detected sturgeon herpes virus I type (Acipenserid herpesvirus 1, White sturgeon herpesvirus type 1) when examining sturgeons in the conditions of recirculating water systems.

Due to little possibilities of the treatment of these fish diseases, the general prophylaxis should be necessary, which includes adequate feeding, elimination of stress factors, which can significantly reduce natural mechanisms of fish protection. The prevention of infectious diseases should be always considered within the framework of fish culture factors. Good sanitary state of aquaculture objects and physical-chemical parameters of water are very important. We proposed a system of treatment and preventive measures, which includes the use of immune modulating agents with strict observance of fish rearing technologies.

An important issue in European countries is reduction of negative environmental impact and water quality improvement by using environmentally friendly drugs in aquaculture. Preparations based on bacteriophages developed by us for treatment of fish bacterial diseases does not negative impact on aquatic organisms compared to antibiotics and chemical drugs and capable of providing significant therapeutic effects.

The studies were conducted using bacteria extracted from different fish species and from water. Fish were taken from different regions of Ukraine. In total, 110 strains of microorganisms were used. Bacteriophages isolated from the strains 0911 (common carp), 0411 (common carp), 5911 (bighead carp) were extracted and accumulated. As a result, 3 strains of bacteriophages were isolated from highly pathogenic bacteria identified as Pseudomonas, and their titer was $7.4*10^6$, $8.3*10^6$, and $7.8*10^6$ BUE / mL. We conducted studies on the application of these bacteriophages for treatment of pseudomonase infection of fish and obtained positive results.

Deterioration of environmental conditions and decrease of water quality results in fish diseases significantly reducing yield of cultured fish and retarding their growth. Therefore, control of environmental conditions of water bodies and development of new environmentally friendly therapeutics is a necessary prerequisite for effective fish culture technologies.

1. Ariel, E., and N. J. Olesen. 2002. “Finfish in Aquaculture and their Diseases – A Retrospective View on the European Community.” Bulletin of the European Association of Fish Pathologists 22 (2): 72–85.
2. OIE (International Office of Epizootics). 2009. Diagnostic Manual for Aquatic Animal Diseases (Chap. 2.3.8.). 3rd ed. Paris: World Organization for Animal Health.

3. Dixon P.F., Ngoh G.H., Stone D.M., Chang S.F., Way K., Kueh S.L.F. 2008 – Proposal for a fourth aquabirnavirus serogroup – Arch. Virol. 153:1937-1941.
4. Golovina N.A, Bauer O.N. 2007 – Ichthyopathology – Mir, Moscow, R448 p. (in Russian).
5. Schelkunov, I. S., and T. I. Schelkunova. 1989. "Rhabdovirus Carpio in Herbivorous Fishes: Isolation, Pathology and Comparative Susceptibility of Fishes." In Viruses of Lower Vertebrates, edited by W. Ahne and E. Kurstak, 333–348. Berlin: Springer-Verlag.

# QUALITATIVE DIAGNOSIS AND EFFECTIVE PREVENTION OF VIRAL INFECTION – KEY FACTORS FOR ANIMAL HEALTH

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Emerging and re-emerging viral diseases – are the main factors, influencing health status of animals, leading to huge numbers of mortality, morbidity and economical loses. Thus, the system of farming, with large numbers of animals in a small area, limits in climate control and a list of other factors, that are present in modern animal's production – provoke the development of infections, especially viral etiology.

Taking into account huge economical background, research in the pathogenesis, diagnostic and prevention of viral diseases of animals – are prerogative directions in veterinary medicine and general virology, supported globally. Diagnosis of viral diseases of animals as well as in medicine, based on direct virus detection, viral antigen detection, virus isolation or viral antibody detection.

However, given the need to maximize rapid results and low cost tests, the most common and is used PCR and ELISA. Currently available a wide range of commercial test systems developed on the basis of these approaches for the diagnosis of certain animal diseases. In addition, a number of scientific centers of the world always continue to work on their development and improvement. The quality of diagnosis and understanding of viral infection - the success of vaccination.

# SESSION
# HUMAN VIRUSES PATHOGENESIS, CHEMO- AND IMMUNOPROPHYLAXIS OF VIRUS DISEASES

# THE PREVALENCE OF DRUG-RESISTANT HIV STRAINS AMONG REPRODUCTIVE-AGED PATIENTS WITH INEFFECTIVE ART IN UKRAINE

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HIV resistance to antiretroviral drugs (ARVs) is one of the main causes of antiretroviral therapy (ART) failure. The development of HIV resistance can potentially compromise future treatment options [1]. Furthermore, antiretroviral drug resistance can limit options for therapy during pregnancy and specific prophylactic of HIV vertical transmission, complicate obstetrical care for HIV-infected women [2].

The research was carried out to study the prevalence of resistant HIV strains in individuals at reproductive age with virological inefficiency of ART.

Blood plasma samples from 42 women and 28 men at reproductive age (average age 33.2 years) with virological failure of ART were tested for the presence of resistant HIV strains. The effectiveness of ART was assessed by the level of viral load (VL) of HIV by at least 6 months after the initiation of therapy or the start of the current treatment regimen. The patients with ineffective ART were included in the study: indicators of HIV VL in the plasma samples of women were ranged from $2.4x10^3$ to $3.18x10^6$ HIV RNA copies/ml. In a group of men – from $4.7\ x10^3$ to $2.55\ x10^6$ HIV RNA copies/ml.

Among woman 22 (52.38±7.64%) were on the first regimen of ART, for 8 women (19.05 ± 5.98%) scheme has changed once, for 12 (28.57±6.90%) it has changed two or more times. Most of the men (20; 71.43±8.47%) have received the first regimen of ART, for 2 (7.14±4.76%) – scheme of therapy has changed twice, for 4 (14.28±6.53%) – it has changed four times.

At the time of investigation all men and 24 women have received ART regimen of 2 nucleoside reverse transcriptase inhibitors (NRTIs)+non-nucleoside reverse transcriptase inhibitor (NNRTI), 18 women received scheme 2NRTIs+1 protease inhibitor (PI).

For the detection of drug resistance mutations in the HIV genome we used the test system ViroSeq®HIV-1 Genotyping System v.2.0 (Celera Corporation, USA). Data analysis and interpretation were performed using Stanford University Database (http://hivdb.stanford.edu).

HIV resistance to ARVs develops as a result of formation of specific mutations in the HIV genome, which leads to changes in the structure of the viral proteins – targets of antiretroviral drugs (ARVs). Samples of blood of HIV-infected patients included in investigation were tested for presence of mutations caused resistance to three main classes of ARVs – NRTIs, NNRTIs and PIs.

In general, among the studied 70 blood samples obtained from women and men with ART failure, the majority (56; 80.0±3.7%) were positive for the presence of HIV strains resistant to antiretroviral drugs. The frequency of detection of mutations resistance to NRTIs and NNRTIs was high – they were determined in 71.43±5.4% and 75.71±5.13% samples, respectively; in 68.57±5.55% samples resistance mutations to two classes of drugs were detected simultaneously. Mutations of resistance to PIs were detected much rarely – in 4.29±2.42% of the samples.

Resistant HIV strains were revealed in 31 (73.80±6.78%) plasma samples of women and 25 (89.29±5.76%) plasma samples of men. NRTIs-resistant HIV strains were found in 27 (64.28±7.39%) plasma samples of women and 23 (82.14±7.16%) plasma samples of men. NNRTIs-resistant HIV strains were in 28 (66.67±7.27%) and 25 (89.29±5.76%) samples respectively. Strains resistant to both NRTIs and NNRTIs were dominant: in general, they were revealed from 25 (59.52±7.57%) plasma samples of women and 23 (82.14±9.56%) plasma samples of men. Among mutations resulted in resistance to NRTIs the substitution M184V was the most frequent (in 77.42±5.34% and 80.0±4.74% plasma samples respectively). In addition to this mutation, in the spectrum of mutations of resistance to NRTIs in HIV strains isolated from women other substitutions were detected with high frequency: the non-thymidine analog mutations K65R (38.71±8.69%), Y115F (22.58±7.44%), L74V/I (19.35±7.02%) and one mutation of the thymidine analogue mutations complex (TAMs) type 2 – K219Q / E (22,58 ± 7,44%). In HIV strains isolated from men, except of the dominant mutation M184V/I, L74V/I mutation and two TAMs type 2 (D67N and K70E) were detected quite often. L74V/I was in 24.0± 8.48% of samples, D67N and K70E met with the same frequency (20.0±7.94%).The frequency of K65R mutation detection in plasma samples of men was 12.0 ±6.42%.

Among mutations caused resistance to NNRTIs, G190S/A was the most common (in 54.84±8.88% and 57.14±6.61% samples respectively).

PIs-resistant HIV strains were found in 3 (7.14±3.97%) women plasma samples and were not revealed in men plasma samples.

Drug-resistant strains of HIV were detected in 73.80% plasma samples of women and in 89.29% plasma samples of men. It was found that the replacements in HIV-1 pol gene, which encodes HIV reverse transcriptase, were dominant. Among them substitutions M184V and G190A were the most frequent. A significant level of K65R mutation prevalence was revealed. K65R provides a reduced sensitivity of HIV to a range of NRTIs, in particular to tenofovir [3]. The obtained data indicate a high level of the prevalence of drug-resistant strains of HIV among people of reproductive age with ineffective ART in Ukraine.


1. Iyidogan P., Anderson K.S. Current Perspectives on HIV-1 Antiretroviral Drug Resistance// Viruses. – 2014. – Vol. 6(10). – P. 4095-4139.
2. Lazenby G.B., Mmeje O., Fisher B.M. et al. Antiretroviral Resistance and Pregnancy Characteristics of Women with Perinatal and Nonperinatal HIV Infection// Infectious Diseases in Obstetrics and Gynecology. – 2016. – 8p.
3. Miller M.D. K65R, TAMs and Tenofovir//AIDS Reviews. – 2004. – Vol.6. – P.22 – 33.


## GENETICAL BASIS OF RESISTANCE TO THE NEURAMINIDASE INHIBITOR AMONG THE UKRAINIAN INFLUENZA ISOLATES


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Influenza, commonly known as flu, is a contagious respiratory viral illness of global importance. The influenza neuraminidase proteins are

highly prone tomutation-relatedchanges[1].Neiraminidaseinhibitors(NAI) are currently most prescribed antivirals for treating influenza infections [2].

The aim of this study was to discoverthe influence that selection-pressurehas played in a neuraminidase (NA) proteingenetic diversity and to screen the genetic changes which associated with reduce susceptibility to NAI in influenza viruses neuraminidase sequences, isolated in Ukraine in 2009-2015 outbreak seasons.

The specimens were collected from patients of sentinel sites in different regions of Ukraine. It was tested for influenza by real-time RT-PCR, and from PCR-positive samples viruses were isolated in MDCK and MDCK-SIAT cellculture.

Phylogenetic analyses were performed using

MEGA 6[3] and BEAST v1.8.1[4] software and were aligned using the ClustalW algorithm. Phylogenetic trees were generated by the neighbour-joining and maximum likelihood methods.

TestsforpositiveselectionwereconductedontheDatamonkeyserver [5] usingthesingle-likelihood ancestor (SLAC), fixed-effects likelihood (FEL), internal branch fixed-effects likelihood (IFEL), mixed effects model of evolution (MEME), and fast unconstrained Bayesian approximation (FUBAR) methods, and the dN/dS ratios were calculated using the SLAC and FEL codon based maximum likelihood approaches.

In the present study, the susceptibility of influenza A(H1N1)pdm09, A(H3N2) and type B viruses to NAIs(oseltamivir, zanamivir and peramivir) is described and examined using a phenotypic fluorescence-based and genotypic-based methodsduring the last six consecutive influenza seasons (from 2009/2010 to 2014/2015). Our findings are in accordance with surveillance data in other countries.

Phylogenetic analyses revealed a high genetic similarity of Ukrainian isolates to viruses strains in other countries. It shows multiple ways influenza viruses import to the country during the monitoring period. Testsforpositiveselection indicated a negligibly sites under positive selection: for the A(H3N2) were revealed positions 93 and 402 in NA protein,for the A(H1N1)pdm – only position 40, for the influenza B Yamagatalineage– positions 74, 99, 268 and for the influenza B Victorialineage– positions 358, 288, 455.Novel amino acid substitutions in the neuraminidase proteins of ukrainian isolates were analyzed.The substitutions which associated with reduce susceptibility to neiraminidase inhibitors (oseltamivir, zanamivir and peramivir) in the majority NA gene of A(H3N2) and A(H1N1) influenza viruses weren't observed in the discovered Ukrainian isolates. Accept only a 2,5%tested A(H1N1)pdmisolates from 2014-2015 influenza season are contain a H275Y mutation which associated with oseltamivirresistance.

To sum up, the genetic drift of influenza viruses in the Ukraine had the similiar direction as in Europe during the 2009-2015 influenza seasons. Our analysis shows that although only a few positions are under positive selection pressure, most of the positions are evolving neutrally or under negative selection. The present study highlights the importance of continued influenza antiviral susceptibility monitoring in clinical specimens.


1. Грип та йогопрофілактика: Навчальнийпосібник/ за ред. Дзюблик І.В., Широбокова В.П. — К., 2005 – 194 с.
2. Nguyen H.T,. Fry A.M., Gubareva L.V. Neuraminidase inhibitor resistance in influenza viruses and laboratory testing methods // AntivirTher. – 2012. – Vol.17. – P. 159-73.
3. Koichiro Tamura, Glen Stecher, Daniel Peterson, Alan Filipski, Sudhir Kumar. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0 // Mol. Biol. Evol. – 2013, Dec. – Vol.30(12): P. 2725–2729.
4. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesianphylogenetics with BEAUti and the BEAST 1.7 //MolBiolEvol. – 2012. – Vol.29(8). – P.1969-1973.
5. Delport W, Poon AF, Frost SD, Kosakovsky Pond SL (2010) Datamonkey 2010: a suite of phylogenetic analysis tools for evolutionary boil


## THE STUDY OF ANTIVIRAL POTENTIAL OF FLUORIDE SUGARS


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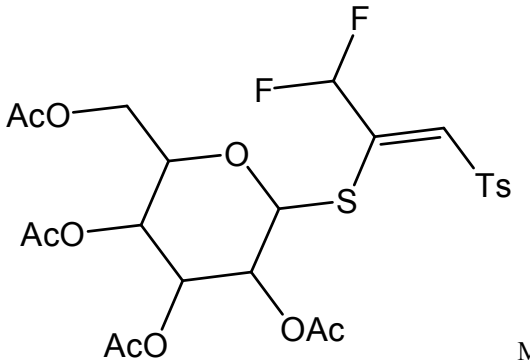
Epstein-Barr virus (EBV) is associated with the pathogenesis of human malignancies such as Burkitt's lymphoma, Hodgkins disease, non-Hodgkin lymphomas, nasopharyngeal carcinoma. A characteristic feature of the virus is its ability to replicate in immunocompetent cells, namely B-lymphocytes, T-lymphocytes, as well as in the epithelium of the upper respiratory tracts. After penetration of EBV into B cells the latter after a

time may initiate unlimited proliferation and eventually may lead to EBV-associated diseases.

The aim was to study the drugs on the development of in Raji and B95-8 cells culture.

Test compounds were thio-S- 1 - (1-n - tolilsulfonil -2-trifluoromethyl – vinyl) -2,3,4,6-tetra-O-acetyl-β-D- glucopyranose (synthesized in Institute of Organic Chemistry of the NAS of Ukraine).




M=594.6

Both compounds showed low toxicity for Raji cell culture and possessed antiviral activity against EBV at low concentrations. Apoptosis progress in infected cells treated with compounds was studied after 3, 24 and 48 hours with DNA-tropic fluorescent dye Hoechst 33342. Uninfected cells and EBV-infected cells both untreated with compounds were used as controls. The percentage of apoptotic cells in controls did not exceed 5%.

The cell lines of B-lymphocytes, which are normally transformed by Epstein-Barr virus (Raji and B95-8) were used. The use of several cell lines allowed us to obtain results about CC50 of the compound and it was 3 μg/ml in the B95-8 cell culture, which produces EBV, and 5 μg/ml in Raji cells.

An antiviral effect of the compounds was determined by real time PCR using appropriate commercial kits. The concentrations that inhibited the reproduction of the virus of 50 percentagewere 1 μg/ml.

So it can be assumed that the test compound possesses the apoptosis induction against both forms of EBV infection and the destruction of cells infected with virus occurs through apoptosis as a result of blocking replication of EBV and therefore its transforming potential. Obtained and analyzed data allows to relate the compound AG-298 to a perspective anti-EBV agent and the apoptosis inducing compounds that can be used in further research on antitumor action.

# ANTIHERPETIC ACTIVITY OF COMPLEX MICROBIAL PREPARATIONS BASED ON METABOLITES of STREPTOMYCETES

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Herpes viral infection is the most common human viral infection. According to the WHO data, about 80% of the world's population has the antibodies to herpes viruses. Modern chemotherapy of infectious diseases, caused by the herpes virus, is associated with the use of guanine-containing drugs, which are the modified acyclic nucleosides of acyclovir, penciclovir, cidofovir and others. The main disadvantage of these drugs, despite their high antiviral activity, is their toxicity and formation of drug resistance. This leads to the necessity for comprehensive study and discovery of the alternative and safe remedies for the prevention and treatment of various forms of herpetic lesions. Among others, the attention of researchers is focused on the compounds derived from natural sources. A promising approach for the treatment of diseases caused by herpes simplex virus is the use of antagonistic microbes and their metabolites. The filamentous soil *Actinobacteria* can be used as a source of the substances with the diverse chemical structures and spectrum of biological activity, and in particular the members of the genus *Streptomyces*, which are widely known as antagonists of pathogens of humans and animals. There are a lot of biologically active substances among their metabolites along with antibiotics, which are enzymes, amino acids, vitamins, lipids, including fatty acids and sterols, lectins, etc. They used for designing of products for medical purposes. Thus the screening of compounds with antiviral activity among *Streptomyces* metabolites is still valid and has practical significance for the development of new antiherpetic drugs.

The aim of this work was to study the antiviral properties of complex microbial preparations that are based on metabolites of *Streptomyces*.

Four microbial biological preparations based on metabolites of soil streptomycetes that were developed at Zabolotny Institute of Microbiology

and Virology of NAS of Ukraine were used in the study. Averkom nova, which is the complex of supernatant of cultural medium (SCM) with ethanol extract from the biomass (EB) of *S. avermitilis* UCM Ac-2179 and with an addition of chitosan. Violar is a mixture of SCM and EB from *S. violaceus* UCM Ac-2191. Multyprotekt is the EB from *Amycolatopsis orientalis* UKM As-2196. Fitovit is the SCM with the EB from *S. netropsis* UKM Ac-2186. In addition the purified fractions of antibiotics complex from *S. violaceus* UCM Ac-2191 were studied too.

The isolation of the active components of the antibiotic complex from *S. violaceus* UCM Ac-2191 was made by primary sorption from the supernatant of culture medium as well as from the extracts of biomasses. The sorption on Amberlyte XAD-2 was followed by the elution with n-butanol-acetone-water (1:1:1) at pH6.8-7.5. Eluates were vacuum evaporated at 37 °C and their dry residues were dissolved in 60% aqueous ethanol. Further purification was performed by thin-layer chromatography (TLC) on the plates of DC-Fertigfolien Alugram SIL G/UV254 Kieselgel 60 (Macherey-nagel) (20Ч20cm). Chromatograms were analyzed under the UV light with densitometer "Sorbfil" (Russia). The fractions, detected after TLC, were isolated, eluted with 60% ethanol and dried at 37 °C. Further they were dissolved in a nutrient medium for cell cultures (50% DMEM + 50% RPMI-1640 (Sigma)) and were used to study their antiviral activity.

Human herpes simplex virus type 1 (HSV-1/US) was used as the viral model. Cytotoxicity and antiviral activity of the preparations were determined in cell culture BHK-21 by standard technique using MTT assay.

Cytotoxic effects of preparations were studied for their 1:8 to 1:1024 dilutions. Violar demonstrated the minimal cytotoxicity in comparison to other preparations and its $CC_{50}$ was 1:16. Multyprotekt, Averkom nova and Fitovit suppressed the cell viability by 50% at the dilutions 1:32, 1:32 and 1:64, respectively.

The non-toxic dilutions of the preparations were used to study their antiviral activity. Showed that all preparations possess the activity against HSV-1/US, but only Violar caused inhibition of viral reproduction by 38%.

Known, that specific components of microbial preparations from streptomycetes may perform different biological activity. Therefore six fractions (F1-F6) were separated from the antibiotic complex of Violar. Their cytotoxicity and antiviral activity against HSV-1/US was studied too.

The purified substances from various fractions were used in concentrations 0.25-0.004 μg/ml to study their cytotoxicity. All studied fractions were non-toxic, considering that the inhibition of cell viability did not exceed 13%.

To study the specific antiviral activity the preparations were used in concentrations 0.125-0.002 μg/ml. Showed that the components of F4

and F5 significantly inhibited the reproduction of HSV-1/US and their $EC_{50}$ were 0.028 and 0.114 μg/ml, respectively. There was not shown the antiHSV-1/US activity for the fractions F1, F2, F3 and F6.

Thus, we showed that microbial preparation Violar that is based on microbial metabolites from soil streptomycetes *S. violaceus* UKM-Ace 2191, as well as some of its components cleared from the antibiotic complex (fractions F4 and F5) possess the antiviral activity and cause inhibition of HSV-1/US reproduction. The data indicate the presence of active antiherpetic components in the composition of Violar that opens the perspective for creation of the new classes of drugs on the basis of streptomycetes metabolites.

## A COMPARATIVE PHYLOGENETIC ANALYSIS OF INFLUENZA VIRUSES A(H1N1)PDM CIRCULATING IN ZHYTOMYR REGION DURING 2009-2015

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Seasonal influenza epidemics impose a heavy burden on society, with 3–5 million cases and 250 000–500 000 deaths worldwide every year. [1].

Traditionally, attention has been directed toward influenza A, which accounts for the majority of influenza cases in most seasons In addition to seasonal epidemics of influenza A virus can also cause a pandemic [2].

Genetic mutation is considered one of the most important molecular mechanisms in the evolution of influenza virus. The influenza virus has low fidelity RNA synthesis, which results in a high mutation rate - around one mutation per genome per replication [3].

There are two main evolutionary mechanisms which allow influenza viruses to constantly evolve and re-infect their hosts, namely, an antigenic drift and antigenic shift [4].

Influenza virus has also shown the propensity to escape immunity because of continuous antigenic drift, i.e., mutation at the epitope positions of HA and NA segments. Antigenic drift may often result in structural changes in antigenic sites, which must be recognized by the host immune system in order to suppress viral infection. This antigenic drift often requires the update of annual influenza vaccines to assure a match between the vaccine and currently circulating viral strains [5].

Thus, the aim of our research was to perform phylogenetic analysis of HA and NA genes of influenza A(H1N1)pdm viruses, which circulated in the Zhytomyr region during the 2009–2015.

Molecular genetics, phylogenetic and statistical methods were used for this study.

The influenza virus A(H1N1)pdm detected in the population of Zhytomyr region in four epidemic seasons: 2009-2010, 2010-2011, 2012-2013 and 2014-2015. In the first three seasons he remained dominant, and last season, there was an epidemic of co-circulation of influenza viruses along with the A(H3N2) and B, but was already dominant influenza virus B. Feature of the epidemic season 2010-2011 was that early in the season flu virus was detected in only, and then only virus influenza A(H1N1)pdm. The result was detected in more clinical material. These results do not coincide with literature data.

**Epidemic season 2009-2010.** This season was different in that it began to spread on the new antigenic properties of influenza virus A(H1N1)pdm. As a result, there was the first pandemic of this century.

Investigated isolates were similar to the vaccine strain A/California/07/2009pdm. Isolates A/Ukraine-Zh/199/2010 and A/Ukraine-Zh/123/2010 became an amino acid substitution M227I, but they had no substitution I321V. All studied isolates were more similar to the reference strain A/Lviv/N6/2009, but not had replacement D222G.

Conducted phylogenetic analysis of nucleotide sequences NA has shown that their volatility is on the same level as in the nucleotide sequences.

**Epidemic season 2010-2011.** It was postpandemic season. Investigated viruses have substitution S185T(A), S451N. Isolate A/Ukraine-Zh/182/2011, together with the reference strain A/St.Petersburg/100/2011 showed substitution S143G and A197T. Isolate A/Ukraine-Zh/60/2011 and gained D97N substitution A186T. Isolates A/Ukraine-Zh/3/2011, A/Ukraine-Zh/60/2011 entered the sixth subgroup and isolate A/Ukraine-Zh/182/2011 entered the seventh subgroup was similar to the reference strain A/St .Petersburg/100/2011.

Investigated isolates epidemic season 2012-2013 and 2014-2015 were as common and group substitution in nucleotide sequences. Isolates two epidemic seasons have become common substitution in nucleotide sequences of genes on D97N, K283E, S185T, E499K. In the nucleotide sequences NA investigated viruses observed substitution V106I.

**Epidemic season 2012-2013.** Isolates of this season have become common substitution V234I, and isolate A/Ukraine-Zh/523/2012 it has. Isolate A/Ukraine-Zh/5932/2013 has substitutions A186T, D274N, and isolate A/Ukraine-Zh/5912/2013 has substitution R327K. These viruses entered subgroup 6C and isolate A/Ukraine-Zh/523/2012, similar to the reference strain A/Hong Kong/5659/2012, entered the genetic group 6A.

The results of phylogenetic analysis of nucleotide sequences NA shown that influenza viruses of this season returned I106V substitution, such as are in the A/California/07/2009, A/Bayern/69/2009, A/England/195/2009. So, in this case there was a reversion. Isolate A/Ukraine-Zh/523/2012 has substitution V106I and like viruses previous seasons 2009-2010 and 2010-2011.

**Epidemic season 2014-2015**. These viruses have replacement K163Q, A256T and were similar to the reference strain A/South Africa/3626/2013. Isolate A/Ukraine-Zh/6786/2015 has substitution D222G, which is also observed in the reference strain A/Lviv/N6/2009. As a result of substitution disease is a difficult course. These viruses entered subgroup 6B.

Phylogenetic analysis of nucleotide sequences NA isolates studied showed that besides the obtained replacements in the epidemic season 2012-2013, acquired new: I34V, I321K, K432E. The Zhytomyr's isolates also have substitutions L40I, I117M, I365T, N386K. Isolate A/Ukraine-Zh/6786/2015 also has substitutions M15V, D416N, and isolate A/Ukraine-Zh/6392/2015 has substitution G454D.

The analysis of the NA genes of the Zhytomyr's region influenza viruses showed similar results with the analysis of the HA gene sequences. All investigated isolates are within the genetic clade A/California/07/2009pdm. However epidemic strains of four seasons in the evolution of different subgroup included in this treasure.

You can also note that the variability of influenza virus A(H1N1)pdm, associated with mutations in genes of surface antigens on NA and viruses is low, indicating that the low probability of the emergence of new variants of the virus, which in antigenic properties very different to from the vaccine strain. Therefore, for the epidemic season 2015-2016 biennium was recommended included in the composition of influenza vaccine a strain A/California/07/2009pdm.

1. World Health Organization. Influenza. Fact sheet no. 211. Available at http://www.who.int/mediacentre/factsheets/fs211/en/ (Accessed 28 March 2014).
2. Epidemiological and virological characteristics of influenza B: results of the Global Influenza B Study / Saverio Caini, Q. Sue Huang, Meral A. Ciblak, Gabriela Kusznierz et al. // Influenza and Other Respiratory Viruses. – 2015. – Vol. 9(1). – P. 3–12.
3. Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution / E. Ghedin, N.A. Sengamalay, M. Shumway, J. Zaborsky, T. Feldblyum et al. // Nature. – 2005. – Vol. 437. – P. 1162–1166.
4. Phylogenetic analysis of influenza A viruses (H3N2) circulating in Zhytomyr region during 2013–2014 epidemic season / O. G. Boyalska, I. M. Kyrychuk, I. G. Budzanivska, A. P. Mironenko et al. // Biopolymers and cell. – 2015. – Vol. 31(3). – P. 226–232.
5. Evolutionary History and Phylodynamics of Influenza A and B Neuraminidase (NA) Genes Inferred from Large-Scale Sequence Analyses / Jianpeng Xu, C. Todd Davis, Mary C. Christman, Pierre Rivailler et al. // PLoS ONE. – 2012. – Vol. 7.(7) – P. 1–15.

# CYTOTOXICITY EFFECT OF FLUORIDE COMPOUNDS OF HETEROCYCLIC ORIGIN

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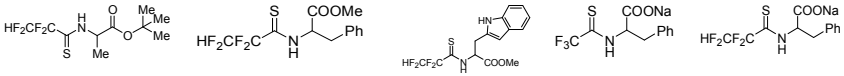
The obligate intracellular parasitic nature of viruses establishes difficulty in the development of antivirals. The main reason behind the limit of antivirals in clinical use is that a number of viral inhibitors target cell metabolism and cause cytotoxic effects.

A study on the cytotoxicity of 6 new fluoride compounds of different origin was held (provided by Institute of Organic Chemistry of the NAS of

Ukraine, prof. Schermolovich**)** due to the significant implication of fluoride analogue compounds of heterocyclic origin in chemotherapy of viral infections.

**10S-20** **10S-21** **10S-22** **10S-23** **10S-24**



Compound 10S-20 belongs to the structural type of tert-butyl ether N-(2,2,3,3-tetrafluorthiopropionyl) alanine. Compounds 10S-21 and 10S-22 belong to the structural type of methyl ether N-(2,2,3,3-tetrafluorthiopropionyl) phenylalanine and N-(2,2,3,3-tetrafluorthiopropionyl) tryptophan. Compounds 10S-23 and 10S-24 are sodium salts of N-(2,2,2-trifluoroacetic) phenylalanine and N-(2,2,3,3-tetrafluorthiopropionyl) phenylalanine. Cytotoxicity effect of compounds were investigated in MDBK (Madin-Darby bovine kidney) cell culture using colorimetric MTT-assay, which measures dehydrogenase activity of mitochondria in living cells and neutral dye method, which indicates the lysosomes' activity. Compounds added to cell maintenance medium and incubated 48 hours. Colometric assay set at 540 nm using Multiscan FC (USA) reader after adding an equal dye.

The results showed, that $CC_{50}$ (cytotoxic concentration, that reduces cell viability by 50%) measured for 10S-22 compound by MTT-assay was equal to 290 μg/mL and by neutral dye method - 554 μg/mL. $CC_{50}$ measured for 10S-23 compound by MTT-assay and by neutral dye method was higher than 1000 μg/mL. Cytotoxic concentrations measured for 10S-20, 10S-21, 10S-24 compounds by neutral dye method were higher than (2022 μg/mL, 1585 μg/mL and 2519 μg/mL, respectively). $CC_{50}$ measured for these compounds by MTT-assay were equal to 653 μg/mL, 394 μg/mL and higher than 1000 μg/mL, respectively.

Therefore, the study of potential antiviral fluoride compounds indicated the difference of cytotoxicity effect due to the level of their concentarations. The level of cytotoxicity effect varied with methods, MTT-assay indicated lower values, which concerns dehydrogenase activity of mitochondria in living cells and neutral dye method indicated higher values, including the lysosomes' activity. The low cytotoxic effect of compounds 10S-20, 10S-21, 10S-23 and 10S-24 raises the prospect of their further research as antivirals.

# DEVELOPING ACCESS TO HEPATITIS C PREVENTION, TREATMENT AND CARE FOR MOST-AT-RISK POPULATIONS IN UKRAINE

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Ukraine belongs to the Eastern Europe region with 69% of viraemic rate of Hepatitis C infection (HCV) [1]. WHO estimates 3% of HCV infection prevalence among general population of Ukraine. HCV prevalence in studies representing general population in Ukraine suggest 12% of people have a history of HCV infection (anti-HCV antibody presence) [2]. At the same time official Ministry of Health records of chronic Hepatitis C (CHC) reports 62 807 cases registered in period between 2010-2014 yrs. Notification and registration of CHC cases in Ukraine started only since 2009. Until present day HCV surveillance remains a major area for development in a system of Public Health. [3] Hepatitis C virus is predominatingly affects people who inject or use intranasal drugs, men having sex with men (MSM), people who were receiving infected blood products in health care facilities with inadequate infection control, medical staff exposed to contacts with untested blood in medical settings, HIV positive people and those who have had a tattoos, piercings. Co-infection HIV/HCV HIV prevalence is 0.59% among adult population. There are 29 890 HIV/HCV co-infected people who are registered in care at HIV/AIDS centers. About 29.0% (n=4532) of HCV and 7.0% (n=1047) of HBV registered among newly detected HIV cases in 2014. The estimated number of people who inject drugs (PWID) is 310,000 with HCV prevalence of 55.0% according to the bio-behavioral study conducted by Alliance for Public Health (Alliance). [4]

Starting from 2009, Alliance has been implementing regular HCV treatment programs among the key population members..

In 2012 Alliance together with its partners initiated a national advocacy campaign “Demand Treatment!”, which includes a component of testing among general population. The campaign contributed to raising public awareness on the routes of transmission and prevention of HCV, mobilizing communities and all stakeholders to counter hepatitis C epidemic, reducing the prices for HCV diagnostic and treatment, developing and approving the State Targeted Social Programs for Viral Hepatitis Prevention, Diagnostics and Treatment and 15 oblast programs, allocating

funds from the state and local budgets, registration of Sofosbuvir in the State Register of Medical Drugs of Ukraine, and launching the first program of HCV treatment, in particular with new direct-acting antivirals (DAAs). Thus, in 2013 Alliance started the first program of HCV treatment with peg-interferon and ribavirin in Ukraine.

Within a pilot project of community supported HCV treatment among key populations sofosbuvir-based treatment regimens were introduced in 2015. The project services include facilitation of the access to HCV diagnostics, treatment and social support. Prevention of HCV reinfection is addressed by 3 months intervention performed by social workers. Treatment efficacy monitoring was established which include demographical diagnostics and clinical data collection.

Over 200 000 HCV rapid tests were performed among people who inject drugs and other most-at-risk populations. Positive results of anti – HCV antibody test range is from 21 – 32% within 2012-2015.

More than 1100 patients received access to sofosbuvir based HCV treatment since the begging of the pilot project.

High cure rates indicate effectiveness of applied community supported approach to provision of medical and social services. It can be illustrated by cohort analysis conducted among 264 patients who initiated treatment within June-December 2015. 73.0% were male, mean age was 38 years old. 246 (96.0%) were HIV/HCV co–infected, 19 (7.0%) were HIV/HCV/HBV co-infected, 10 (4.0%) were HCV mono infected. 56 (23.0%) out of HIV/HCV co-infected had TB treatment history. Key populations were presented primarily by PWID - 220 (86%), 14 (5.0%) patients were on opioid substitution therapy (OST), 22 (8.0%) were represented by commercial sex workers (CSW), men who have sex with men (MSM) and other risk populations. Fibrosis stages distribution: F1 - 60 (23.0%), F2 – 87 (34.0%), F3 – 52 (20.0%), F4 – 56 (22.0%). The prevalent genotype was G1 (48.0%), followed by G3 (42.0%), G2 (9.0%) and G4 (1.0%). SOF+PegIFN+RBV (12w) was prescribed in 82.0% of patients, SOF+RBV (12 w) – in 9.0% and SOF+RBV (24 w) in 8.0% of patients. Overall 240 patients achieved SVR 12 (91.0%), 16 (6.0%) had relapse and 8 (3.0%) dropped out (2 adverse events, 4 refused treatment, 2 death). Patients with G1 achieved 90.0% SVR12, with G2 80.0%, G3 95.0% and G4 100% SVR 12 rates. Treatment response in non-cirrhotic and cirrhotic patients is 93.0% (n=191) SVR12 versus 86.0% (n=51).

Sofosbuvir-based HCV treatment regimens showed high SVR 12 rates among hard-to-reach high risk populations; primary HIV/HCV co-infected PWID. Dropout rates due to discontinuation of treatment are very low. Role of social/peer support services during and after the treatment should be studied further as service model in a standard HCV care for hard-

to-reach populations. On the country policy level Ukraine requires development of comprehensive Hepatitis C elimination strategic plan which will cover all aspects from surveillance to the access to affordable diagnostics and treatment.


1. Guidelines for the Screening Care and Treatment of Persons with Chronic Hepatitis C Infection. Geneva: World Health Organization; 2016 (http://apps.who.int/iris/bitstream/10665/205035/1/9789241549615_eng.pdf)
2. Hope V.D., Eramova I., Capuro D., Donoghoe M.C. Prevalence and estimation of Hepatits B and C infections in the WHO European Region: a review of data focusing on the countries outside the European Union and the European Free Trade Association. Epidemiol. Infect. (2014), 142, 270–286.
3. Global Policy Report on the Prevention and Control of Viral Hepatitis in WHO Member States. Geneva: World Health Organization; 2013
4. Behavior Monitoring of injection drug users as a component of second generation surveliance. Kyiv: Interational HIV/AIDS Alliance in Ukraine; 2013

# *IN VITRO* ANTIVIRAL ACTIVITY OF A HERBAL DRUG PROTEFLAZID AGAINST EPSTEIN-BARR VIRUS

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Proteflazid is a herbal drug active components of which are oxygen-containing heterocyclic flavonoid glycosides. It is a domestic product used in clinical practice as antiviral and immunomodulatory drug. The aim of the study was to study an antiviral activity against Epstein-Barr (EBV) of Proteflazid. EBV is the etiological agent of infectious mononucleosis and is associated with a number of lymphoproliferative and autoimmune diseases.

Study was performed with EBV-positive human B-cells (Raji) – latently infected with EBV, and EBV-producing tamarin (*Saguinus oedipus*) B-cell line (B95-8). Cytotoxicity of the Proteflazid was studied using MTT-method and inhibition concentrations of the drug that inhibited 10% ($IC_{10}$) and 50% ($IC_{50}$) of cell viability were determined. $IC_{10}$ were 16 and 8 μg/ml, $IC_{50}$ were 36 and 25 μg/ml for Raji and B95-8 cells respectively. An antiviral activity of the Proteflazid was measured by semiquantitative PCR in EBV infected cells Raji, as a model of acute infection and in non-induced B95-8 cells as a model of chronic EBV infection. The drug effectively inhibited replication of the virus in the studied concentration range from 0.01 to 8 μg/ml in Raji cell culture. Effective concentration of the Proteflasid that inhibited 50% level of viral DNA accumulation ($EC_{50}$) was 0.02 μg/ml, $EC_{90}$ (90% inhibition level of EBV DNA replication) – 1.8 μg/ml in Raji cells, while on the model of chronic infection drug did not show antiviral activity.

Some viruses, including the EBV, have mechanisms for blocking apoptosis of infected cells, which can lead to malignant transformation and development of tumors. Due to the properties of the virus, we examined the ability of the Proteflazid to induce apoptosis under conditions of the latent

and chronic EBV infection. Apoptotic cells were detected by flow cytometry after treatment with DNA staining propidium iodide hypotonic solution.

Ability of the Proteflazid to induce apoptosis in cell cultures was studied. Proteflazid at the non-toxic concentration 5 μg/ml induced 25% apoptotic cells in Raji cell culture after 48 h incubation, and at the concentration 30 μg/ml, which was close to the $IC_{50}$ index, – 74%. Induction of apoptosis was observed in cells B95-8 under influenced Proteflazid. The drug at the concentration 5 μg/ml induced 33%, and at the concentration of 30 μg/ml - 67% of apoptotic cells after 48 h incubation.

Based on the data we concluded that the herbal drug Proteflazid revealed antiviral activity in acute EBV infection and induced apoptosis of lymphoblastoid EBV-positive cells which is one of the way of the virus-infected cells eliminating.

## ANTIVIRAL ACTIVITIES OF IODINE AND SELENIUM NANOFORMULATION AGAINST SELECTED DNA AND RNA VIRUSES

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Rhabdoviridae family includes about 80 species that infect mammals, fish, mosquitoes and plants. For human pathogens are vesicular stomatitis virus and Lyssavirus. Vesicular stomatitis virus pathogenic for cattle, horses, pigs and humans. Transmitted by mosquitoes in animals, as well as in closed collectives through airborne droplets and through saliva. In most cases of vesicular stomatitis virus is common in people who work with animals. The problem of causal treatment has not been solved.

The relative in vitro antiviral activities of I and selenazole compounds were studied against selected DNA and RNA viruses.

Antiviral compound (iodine and selenazole) was synthesized by Kaplunenko V.G.

Culture BHK21 cells (syrian hamster kidney) and the PTP (testicles pig cells), herpes simplex virus type 2 (HSV-2), Vesicular stomatitis virus (VSV) strain Indiana were obtained from the bank of cell cultures and viruses DK Zabolotny Institute of Microbiology and Virology NASU. The BHK21 or PTP cells were cultured into 96-well culture plates provide separately cytotoxicity assay and antiviral assay.

*Antiviral assay*: Cells in the plates were infected with a proper predetermined 50% tissue culture infective dose of virus (0.1 ml per well). After virus adsorption, the test compounds were added to each well in duplicate manner. Duplicate wells were used for evaluation of antiviral activity, and single wells were used for determination of cytotoxicity. The degree of inhibition of virus-induced CPE and compounds cytotoxicity was observed microscopically after 72 h of incubation. The 50% effective dose (ED50) values were scored microscopically after Microplate Readings and double-checked from the crystal-violetstained 96-well plates.

*Results*: We have shown that I and selenazole have low toxicity; CC50 of the compounds to cause death to 50% of PTP and BHK21cells were accompanied which their cytotoxicity.

The degree of inhibition of the viral CPE on PTP cells were respectively 2,0 and 1,5 lg.

Based on these data, we can conclude that the preparation of iodine and selenazole antiviral agent is active against an RNA-containing virus of the family Rhabdoviridae. It offers the prospect of his research on models of RNA viruses from other families. In contrast, HSV-2 was not susceptible to the inhibitory action of iodine and selenium nanoparticles citrate.

# ANTIVIRAL ACTIVITY OF COBALT, SILVER AND GOLD NANOPARTICLES CITRATE

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Nanoparticles (NPs) of some metals, with their high bioavailability and low toxicity, have attracted wide attention for their anti-viral properties. Nano aqua-chelates are the most interesting new class of complex compounds which prevents the risk of oxidative damage in animal tissues.

The goal of the work was to investigate the antiviral activity of metalsmicroelements NPs, cobalt (Co), silver (Ag) and gold (Au) in respect of a DNA-containing virus - human herpes simplex virus type 2 (HSV-2).

The solution metalsmicroelements NPs were sterilized by passage through a 0.22-,um membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.). Culture Vero cells and virus were obtained from the bank of cell cultures and viruses DK Zabolotny Institute of Microbiology and Virology NASU. Antiviral compounds (metal NPs) were synthesized by Kaplunenko V.G. Vero cells were cultured into 96-well culture plates provide separately cytotoxicity assay and antiviral assay. Antiviral assay: Cells in the plates were infected with TCD50/0.1 ml per well. After virus adsorption, the test compounds were added to each well in duplicate manner. Duplicate wells were used for evaluation of antiviral activity, and single wells were used for determination of cytotoxicity. The degree of inhibition of virus-induced CPE and compounds cytotoxicity was observed microscopically after 72 h of incubation. The 50% effective dose (ED50) values were scored microscopically after Microplate Readings and double-checked from the crystal-violetstained 96-well plates.

Results: We have shown that cobalt NPs and silver NPs have lower toxicity than gold NPs; CC50 of the nanoformulation to cause death to 50%

of Vero cells were accompanied which their cytotoxicity. The degree of inhibition of the viral CPE by Co, Ag and Au NPs were respectively 2,0; 1,0 and 1,5 lg. Further cobalt NPs is found to inhibit 100 times the reproduction of the HSV-2.

We found that the compounds of Co, Ag and Au NPs are inhibitors effective against the reproduction of HSV-2, so they might be useful for treating of herpes simplex virus infections.

## EFFECT OF OLIGORIBONUCLEOTIDES-D-MANNITOL COMPLEX ON THE HEMAGGLUTININ OF INFLUENZA A(H1N1) VIRUS

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The influenza (flu)A viruses cause major respiratory tract infections in humans birds and lower mammals that are responsible for many deaths and great economic losses every year. The flu virus surface glycoprotein hemagglutinin (HA) is responsible for viral attachment to sialic acid-containing host cell receptors and it facilitates the initial stage of viral infection [1].

It is known that natural and synthetic oligoribonucleotides (ORNs) have a wide range of biological activities and can be a used in antiviral treatment since they play a key role in antiviral activity and can change a conformation of some proteins. In our previous study was shown that complex of ORNs with D-mannitol (ORNs-D-mannitol) has higher antiviral activity than ORNs [2]. However, the mechanism of ORNs-D-mannitol antiviral activity is still not clear. So, the **aim** of the present research was to study effect of the ORNs-D-mannitol on the HA activity off lu virus and interaction between them.

The objects of the study were flu A virus (*A/FM/1/47(H1N1))* and its HA protein. The hemagglutinin (HA) activity of flu virus was studied by HA assay after flu virus incubation with ORNs-D-mannitol. Virus control

was the flu virus without such incubation. To investigate interaction of HA protein at the presence and/or absence of the ORNs-D-mannitol fluorescence spectroscopy was used.

It was shown that the ORNs-D-mannitol decreases the HA activity of flu virus by 4 times after incubation of virus with the ORNs-D-mannitol in comparing to the virus control.

Decreasing of the fluorescence intensity of HA of flu virus at the presence of the ORNs-D-mannitol was observed. This effect may indicate on the interaction between the HA and ORNs-D-mannitol that causes conformation changes of HA. For further verification of this assumption we used fluorescence data to calculate dissociation constant that appeared to be relatively weak (micromolar) in our case ($k_d$= 4.91μM).

Our research demonstrates that the complex of ORNs-D-mannitol binds to HA of flu virus and in this manner reduces HA activity. It let us to assume that one of mechanisms of ORNs-D-mannitol antiviral activity may be the changes of HA conformation.

1. Kwon H.M. An RNA aptamer that specifically binds to the glycosylated hemagglutinin of avian influenza virus and suppresses viral infection in cells / H.M Kwon, K.H Lee, B.W Han et al.//PLoS One. – 2014. – Vol. 9, №5. − P.97574.

2. Tkachuk Z.Yu. (2013) Pat. US8420617 B2, US 13/046,240. Multi-antivirus compound, composition and method of treatment of virus diseases / Owner name: BIOCELL LABORATORIES, DISTRICT OF COLUMBIA; Effec. 2011.03.10; pub. 2013.04.16.

## THE LATEST NEWS ON HIV PREVENTION AND DIAGNOSIS

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The modern HIV prevention approach includes evidence-based behavioral programs, pre-exposure prophylaxis (PrEP), and post-exposure prophylaxis (PEP). Behavioral programs focus on HIV transmission risk reduction, voluntary HIV testing and post-test linkage to HIV care for those who tested positive. PrEP aims to support individuals who may be

frequently exposed to HIV, promoting regular medication intake (Truvada) to lower chances of becoming infected. Lastly, PEP is reserved for emergency situations, preventing HIV infection after a possible recent exposure. It involves taking two or more HIV medicines from one or multiple drug classes within 3 days after the high-risk event to stop HIV replication.

Since the U.S. Food and Drug Administration (FDA) approved the OraQuick® In-Home HIV test, self- testing has emerged as a novel strategy to overcome stigma, perceived discrimination and fear of social visibility that discourage individuals from taking tests for HIV infection in health care facilities. The studies have showed that HIV self-tests are generally accurate and simple, can be performed with minimal training. Though, their reactive (preliminary positive) results require confirmatory testing, during which serum or plasma specimens are sent to an HIV laboratory. In addition, HIV self-test positive/ negative predictive values can be affected by the prevalence of HIV in the population served.

## THE IMPACT OF THE DRUG BASED ON RIBONUCLEIC ACID RELATIVE TO EPSTEIN-BARR VIRUS

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Within the last decades ever more attention is given to the creation of preparations for pathogenetic therapy with polyvalence pharmacological operating that are capable to essential influence on the mechanisms of inflammation and immunity, to regulate the main exchanging processes in organism etc. This problem is actual according to the one of representatives of the *Herpesviridae*family, Epstein-Barr virus. Despite of the large number

of antiherpetic drugs, only a few of them show the activity against given virus.. An infectious mononucleosis is considered the clinical form of primary EBV infection of human after that the virus is retained in human organism during all life with the subsequent reactivation under influencing of the different factors, both internal condition of an organism, and environment that results in clinical presentations of a miscellaneous degree of complication. EBV can be the agent to cause miscellaneous lymphomas such as nasopharyngeal carcinoma, carcinoma of parotid glands, stomach adenocarcinoma and other diseases. As well as other herpesviruses EBV affects central and peripheric nervous systems. Thus, the EBV-infection is significant for miscellaneous areas of medicine specifically infectology, infectious neurology, transplantology, hematology.

The purpose of our work was studing of antiEBV activity of drug based on ribonucleic acid in a system in vitro. Researches were conducted in the limphoblastoid cell line Raji (Human Burkitt's lymphoma cell line) and B95-8 (Monkey Burkitt's lymphoma cell line) that allows modeling on acute process of development of EBV-infection. To study the cytotoxicity of investigated drugs they were entered into the culture of not infected cells in concentration from 1 up to 5000 μg/ml. In 48 hours there was conducted the MTT-analysis of the investigated samples. It was shown, that the concentration that oppressed proliferative activity of cells on 50 % ($CC_{50}$) for has compounded 5000 μg/ml (Raji). The anti-virus activity was determined by a PCR method, using "AmplySens 100 R-FL" system (Russia). Drugs were investigated in concentrations of 10-500 μg/ml. The analysis of obtained data allowed to determine concentrations, which oppressed the replication of the virus, that was shown by reduction of the number of genomic equivalents of EBV DNA on a cell testified. $EC_{90}$ for Raji cells was 50 μg/ ml. Study of virucidal action of the tested substance (30 min exposure) allowed to determine the $EC_{50}$ dose which was 200 μg/ml. The analysis of the antiviral activity of the investigated drug in cells chronically producing EBV showed its high efficiency at the level of 50 ug/ml. Based on the preliminary results of study of binding of purified viral capsid proteins to tested drug by Mass spectrometric, we can conclude that its structure influence on surface epitopes of the virus and thus inhibit cell infection process.

Thus, proceeding from the low toxicity of investigated drugand the index of selectivity (100), it is possible to make a conclusion about its availability for the further researches as of drug that are active against an Epstein-Barr virus.

# ANTIVIRAL AND IMMUNOSTIMULATORY POTENTIAL OF FLUORINE CONTAINING TRIAZOLES

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The search of effective antiviral drugs is a scientific problem concerned with a high morbidity and wide spread of viral infections. The nucleosides, modified in heterocyclic, phosphate or carbohydrate fragment of their molecule are used in most medical cases for the treatment of herpes infections. The purpose of this study was to evaluate the anti-herpetic activity of fluorinated nucleoside compounds G8 and G9 (2-N-substituted-4-tosyl-5-polyfluoroalkyl-1,2,3-triazole) in *in vivo* models and determine their immunomodulatory potential.

Previous *in vitro* studies regarding herpes simplex virus type 1 revealed that both compounds are good anti-herpetic agents with the selectivity indexes 18 and 130. This paper presents results of research of anti-herpetic activity studied in the model of herpes meningoencephalitis (in mice) and compared to the reference drug acyclovir. Evaluation of drug activity performed by comparing mortality in experimental and control groups, determining the percentage of animal mortality. The dynamics of animal deaths in the experimental groups was recorded daily throughout the experiment. In the virus control group it was observed on the 4, 6, 10 and 14 days, indicating the specific virus induced death of animals. Shown a significant inhibition of virus reproduction under the action of the compounds at concentrations 0.4 and 0.5 mg/kg, which was more effective of acyclovir. The protection ratio was 80%. In experimental animals the levels of cytokines in the serum were analyzed after the action of the compounds on the virus infected and uninfected animals. The IFN-γ production is the most rapid respond to the virus infection; therefore the immunomodulatory potential of nucleoside compounds was checked by the levels of IFN-γ and two pro- and anti-inflammatory cytokines IL-2 and IL-

4. Observed the increased levels of IFN-γ and IL-2 in the serum of animals that indicates the immunomodulatory effect of the fluorinated nucleoside compounds. Our study showed that fluorine containing triazoles possess the anti-herpetic and immunomodulatory activities and therefore the more deep researches of the mechanisms of their action have to be carried out.

## RBC MEMBRANES FATTY ACID COMPOSITION ISN'T ALTERED IN MICE DURING ACUTE HSV-1 INFECTION IN COMBINATION WITH CERIA DIOXIDE NANOPARTICLES TREATMENT

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The ratio between omega-3 and omega-6 fatty acids (FAs) in organism's tissues may indicate whether it undergoes inflammation because they are the precursors of oppositely acting classes of eicosanoids regulating the immune response. Previous studies showed the change in RBC fatty acids composition during chronic HSV-1 infection in mice and human [1], however, there is no data on this parameter in acute herpetic infection settings.

The purpose of our study was to investigate RBC membranes fatty acid composition on the mice model of herpetic meningoencephalitis in combination with the multiple per-oral administration of perspective antioxidant agent – ceria dioxide nanoparticles (CNP) [2]. We used Balb/c mice weighing 10-12 g with n=6 in each of 4 groups: control, HSV-1-alone, CNP-alone, HSV-1+CNP. The experiment lasted for 8 days during which treatment groups received CNP intraday dose 1 mg/kg. Virus groups were infected IC with HSV-1 strain L-2 in dose ~1 LD100 on 4th day. RBC membrane fatty acids were extracted using modified Folch technique from blood samples collected on 8th day. Analysis of FAs composition was carried out on Agilent 6890N/5973 inert GC/MS system. We identified and measured relative content of 13 FAs in all samples using FAMQ-005

standard solution. Intergroup differences were considered to be significant at $p < 0.05$ when analyzed by Mann-Whitney test. Comparing median +/- interquartile FAs percentage values we found no statistical differences between control and test groups. It may suggest that RBC membrane FAs composition isn't labile enough to undergo noticeable changes during the short period of acute viral infection or eicosanoids aren't actively involved at that stage of infection.

This finding may be one of the explanations why we couldn't observe CNP realizing its activity. Hence, we anticipate chronic HSV-1 infection in mice model more appropriate for further research of antiherpetic CNP activity.

1. N.A. Ishutina, Composition of polyunsaturated fatty acid in the blood of the navel in herpes-virus infection contamination. The medical journal of the Far East, №2, 2013, p. 49-51.
2. Shcherbakov A.B., Zholobak N.M., Spivak N.Ya., Ivanov V.K., "Advances and Prospects of Using Nanocrystalline Ceria in Prolongation of Lifespan and Healthy Aging", Russ. J. Inorg. Chem., 60:13 (2015), 1595–1625

## RESPONSE OF INTERFERON SYSTEM TO INTRODUCTION OF RECOMBINANT INTERFERON MODIFIED WITH CERIUM DIOXIDE NANOPARTICLES

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Interferons (IFNs) are cytokines produced and secreted by virtually all cells of vertebrates in response to stimulation by certain viruses, bacteria,

antigens, and mitogens; they mediate their biological activity by binding to specific receptors on the cell surface [1]. Due to prospect and active using the recombinant interferon alpha-2β (rIFN α-2β) in antiviral and anticancer treatment [2, 3, and 4], it is the important question to enhance its pharmacokinetic properties and sustained efficacy of antiviral action. In this case, many research highlights pegylated rIFN α-2β with better pharmacokinetic properties [5, 6]. Nanocrystalline cerium dioxide (NCD) is a very promising material for biomedical applications [7] and particularly in antiviral therapy [8]. Also, it was shown the increasing immunogenicity of the influenza vaccine by NCD modification [9] and activity of interferon *in vitro* [10]. Thus, NCD can be perspective material in increasing of rIFN α-2β activity. In view of the above, the aim of our work was to study the response of interferon system to injection of recombinant rIFN α-2β modified with cerium dioxide nanoparticles.

In our study, we were using recombinant interferon alpha-2β (Laferobionum, Ukraine) in 1 000 000 IU/ml and nano-ceria aqueous sol containing no stabilizer ("naked" ceria nanoparticles) synthesized by the method proposed by Ivanov *et al.*, which comprises microwave hydrothermal processing of the precursor $CeO_2$ sol formed by the anionite treatment of cerium (III) nitrate aqueous solutions. According to transmission electron microscopy (TEM) data, mean $CeO_2$ particle size in this sol is about 6 nm; the particles are well-crystallized and have an octahedral shape [11]. The white mice were injected by 100 IU/ml of rIFN α-2β intraperitoneally. Serums from mice have been gotten on 3-d, 5-th, 7-th and 14-th days and activity of interferon in samples was tested using microtitration method [12]. The method is based on the studied serums' ability to protect the reference cell line of murine fibroblasts L929 (L929-cells; from the collection of the R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, NASU) against cytopathic effect of *Vesicular stomatitis virus* (VSV, *Rhabdoviridae*, Indiana strain from collection of D.K. Zabolotny Institute of Microbiology and Virology, NASU of Ukraine). Briefly, the cells were cultured in 96-wells Sarstedt-plates using 199 medium (Sigma, USA) supplemented with 7% fetal bovine serum (FBS, Sigma, USA), 100 units/ml gentamicin (Arterium, Ukraine) and 50 units/ml kanamycin (Arterium, Ukraine) in order to form cell monolayer under the stable $CO_2$ level. Cells were maintained at 37˚C for 24 hours in TC-80M-2 incubator with 5% $CO_2$ at a humidified atmosphere. The studied serum samples were added to the cell culture (20 µl serums to 180 µl cell medium with gradual twofold dilution). Each sample dilution was analyzed in three times. In 18–20 h incubation, the studied samples were moved away and 0.1 ml test virus suspension (100 $TCD_{50}$) was added. The wells containing only cell growth medium were used as cell control

ones, the wells with the formed monolayer containing the virus dose only were used as a virus control. The delay of VSV cell destruction effect development was considered as a ratio of the quantity of living cells to the control uninfected ones considering the cell destruction effect in the virus control holes. The obtained results were considered only in case of absence of cell destruction in the cell control samples and all cell degeneration in virus control ones. The maximum sample dilution able to cause the cell destruction virus effect was considered as the interferon titer. We used the WHO International Standard Interferon Alpha 2b (Human rDNA derived) NIBSC code: 95/566 as a positive control. The activity of interferon is calculated as the ratio of the sample titer value multiplying by the activity of the standard sample to the standard titer value. The interferon activity was expressed in international units per milliliter of serum samples (IU/ml).

In the research, we studied the response of interferon system of mice after single injection of rIFN α-2β (IFN-group) in 100 IU/ml and equivalent injection of modified with NCD rIFN α-2β (IFN-NCD-group). On the 3-d day, there was a little response to interferon in IFN-group than equals 50 IU/ml. In the IFN-NCD-group there wasn't any activity of interferon. The specific activity of interferon in the IFN-group on a 5-th day was at the same level with the 3-d day, but the activity of interferon in IFN-NCD-group increased to 200 IU/ml. The top of interferon response in both groups was on the 7-th day. The specific activity in IFN-group and IFN-NCD-group were 100 IU/ml and 800 IU/ml respectively. On the 14-th day, there was established the decrease of interferon levels. In IFN-group specific activity of interferon was 25 IU/ml and in IFN-NCD-group – 100 IU/ml.

The results showed that modified with NCD rIFN α-2β caused enhanced interferon response in mice in comparative with non-modified rIFN α-2β. It is the point, that response the interferon system to the introduction of modified with NCD rIFN α-2β was later than to non-modified rIFN α-2β, but the specific interferon system activation of its was eightfold higher. In the study, there were proved the increasing of interferon level in the serum of mice after single intraperitoneal injection of modified with NCD rIFN α-2β. But, there is the question of establishing mechanisms of this influence and this is the subject of feather researches.

1. Structural and biological characterization of pegylated recombinant interferon alpha-2b and its therapeutic implications / [Y. Wang, S. Youngster, M. Grace *et al.*]. // Advanced Drug Delivery Reviews. – 2002. – №54. – P. 547–570.

2. Lin F. Interferons: Success in anti-viral immunotherapy / F. Lin, H. A. Young. // Cytokine & Growth Factor Reviews. – 2014. – №4. – P. 369–376.

3. Long-term outcome of chronic hepatitis C after sustained virological response to interferon-based therapy / [C. Koh, T. Heller, V. Haynes-Williams *et al*]. // Alimentary Pharmacology and Therapeutics. – 2013. – №37. – P. 887–894.

4. Ningrum R. A. Human interferon alpha-2b: A therapeutic protein for cancer treatment / Ratih Asmana Ningrum. // Scientifica. – 2014. – P. 1–8.

5. A pharmacokinetic and pharmacodynamic comparison of a novel pegylated recombinant consensus interferon-α variant with peginterferon-α-2a in healthy subject / [L. Zheng, M. P. Li, Z. P. Gou *et al*]. // 2015. – №79. – P. 650–659.

6. Pharmacokinetics, tissue distribution, excretion, and antiviral activity of pegylated recombinant human consensus interferon-α variant in monkeys, rats and guinea pigs / [Y. Cai, Z. Zhang, K. Fan *et al*]. // Regulatory Peptides. – 2012. – №173. – P. 74–8.

7. Nanocrystalline ceria based materials – Perspectives for biomedical application / [A. B. Shcherbakov, V. K. Ivanov, N. M. Zholobak et al]. // Biophysics. – 2011. – №56. – P. 987–1004.

8. Antiviral effectivity of ceria colloid solutions / [N. Zholobak, A. Shcherbakov, V. Ivanov *et al*]. // Antiviral Research. – 2011. – №90. – P. A21–A78.

9. Cerium dioxide nanoparticles increase immunogenicity of the influenza vaccine / [N. M. Zholobak, A. P. Mironenko, A. B. Shcherbakov *et al*]. // Antiviral research. – 2016. – №127. – P. 1–9.

10. Effect of nanocrystalline cerium dioxide on the activity of the drug Laferon Farmbiotek / [N. M. Zholobak, L. D. Kryvochatskaya, A. B. Shcherbakov *et al*]. // Proc. of Reports. Conf. Nanotechnology in Oncology. – 2010. – P. 44–48. – *in Russian*.

11. Microwavehydrothermal synthesis of stable nanocrystalline ceria sols for biomedical uses / [V. K. Ivanov, O. S. Polezhaeva, A. B. Shcherbakov *et al*]. // Russian Journal of Inorganic Chemistry. – 2010. – №55. – P. 1–5.

12. Rubinstein S. Convenient assay for interferons / S. Rubinstein, P. Familletti, S. Petska. // J. Virol.. – 1981. – №37. – P. 755–758.

# GENETIC ANALYSIS OF H3N2 INFLUENZA VIRUSES ISOLATED IN UKRAINE IN 2015-2016 EPIDEMIC SEASON

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The hemagglutinin (HA)is surface glycoprotein of influenza viruses, the major target for neutralizing antibodies. Point mutations in the potential antigenic domains of this protein are thought to allow viruses to evade established immune antibodies in the human population. Some influenza seasons are more severe than others, and this is thought to be a reflection of the degree of antigenic change in the HA protein of newly appearing antigenic variants from those of the former strain. Viruses with antigenically drifted HA proteins thus have a selective advantage in becoming the subsequent epidemic strain. Analyses of epidemic influenza virus isolates, therefore, have chiefly focused on antigenic characterization of the HA glycoprotein in order to detect new variants of each epidemic strain for the recommendation of vaccine strains in each season. Although there have been a number of reports on the characterization of human H3N2 influenza viruses, it was recently reported that detection of a new H3N2 antigenic variant is often associated with the rapid disappearance of the former variant [1].

There are 7 genetic groups of influenza A(H3N2) viruses today, but only 3 of them are circulating around the world [2].

In 2012-2013 season Ukrainian isolates belonged to two subclusters 3A and 3C, but since 2013-2014 season only 3C group isolates have been circulated. This group has three subdivisions: 3C.1, 3C.2 and 3C.3 and three new genetic subgroups have emerged in 2014, one in subdivision 3C.2, 3C.2a, and two in 3C.3, 3C.3a and 3C.3b. Viruses in genetic subgroup 3C.2a were the most common. Many viruses in subgroup 3C.2a did not agglutinate red blood cells [3].

Nasal-throat swabs taken from influenza-affected patients from different regions of Ukraine, collected during 2015-2016 years, were used in the study. Samples were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses were isolated in MDCK and MDCK-SIAT cell culture.

Sequencing of influenza viruses genes, isolated in our laboratory, was performed in the World Influenza Center in London using the technology of RNA-SEQ, which allows to sequence coding and noncoding mRNA. Nucleotide sequences were translated into amino acid sequences using MEGA 6 software [4].

All investigated isolates from Ukraine and other countries had several common amino acid substitutions, characteristic A/Victoria/208/2010 cluster, like K62E, T212A and S214I.

All Ukrainian isolates in 2015-2016 season belonged to genetic group 3C.2a. Amino acid substitutions that define these subgroup are F159Y, K160T (resulting in the gain of a potential glycosylation site), V186G, N225D and Q311H.

In Ukrainian isolates were observed point substitutions; A/Kyiv/314/2016 had three mutations – H18Y, I192T and K207R. Isolate from Dnepropetrovsk had substitutions Q197K and G263E.

Molecular genetic features of influenza A/H3N2 virus strains isolated in Ukraine in 2015-2016 season are similar to those of the vaccine strain A/Switzerland/9715293/2013 despite a number of unique amino-acid changes. However, isolated viruses were more similar to the new vaccine strain A/Hong Kong/4801/2014.

1. Mostafa A. Phylogenetic analysis of human influenza A/H3N2 viruses isolated in 2015 in Germany indicates significant genetic divergence from vaccine strains / A. Mostafa, S.M. Abdelwhab, H. Slanina, M.A. Hussein, I. Kuznetsova, C.G. Schьttler, J. Ziebuhr, S. Pleschka // Archives of virology. – 2016. – Vol.161. – P.1505-1515.
2. World Health Organization Influenza Centre. NIMR interim report.2014:1-95.
3. World Health Organization Influenza Centre. NIMR interim report.2015:1-98.
4. Tamura K. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0 / K. Tamura, G.Stecher, D. Peterson, A.Filipski, S.Kumar //MolBiolEvol. – 2013. – Vol.30 (12). – P.2725–9.

# ROLE OF NS1 INFLUENZA A(H1N1)PDM09 PROTEIN AS A VIRULENCE FACTOR

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The non-structural (NS1) protein of influenza A viruses is a non-essential virulence factor that has multiple accessory functions during viral infection. In recent years, the major role ascribed to NS1 has been its inhibition of host immune responses, especially the limitation of both interferon (IFN) production and the antiviral effects of IFN-induced proteins [1].

Nasal-throat swabs taken from influenza-affected individuals in different regions of Ukraine during outbreak were used in the study. Samples were analyzed using real-time polymerase chain reaction (RT-PCR). Phylogenetic trees were constructed using MEGA 6 software [2].

Sequences of influenza viruses from other countries were received from GISAID resource [3], using BLAST (Basic Local Alignment Search Tool) analysis.

Amino acid substitutions D2E, N48S, and E125D were identified in the NS1 protein in 2015-2016 epidemic season. 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.

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 [4].

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.

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 [4].


1. Hale B.G. The multifunctional NS1 protein of influenza A viruses / B.G. Hale, R.E. Randall, J. Ortнn, D. Jackson // The Journal of general virology. – 2008. – Vol.89. – P.2359-2376.
2. Tamura K. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0 / K. Tamura, G. Stecher, D. Peterson, A. Filipski, S.Kumar //MolBiolEvol. – 2013. – Vol.30 (12). – P.2725–9.
3. Режим доступу:http://platform.gisaid.org/
4. Komissarov A. Rapid spread of influenza A(H1N1)pdm09 viruses with a new set of specific mutations in the internal genes in the beginning of 2015/2016 epidemic season in Moscow and Saint Petersburg (Russian Federation) / A. Komissarov, A. Fadeev, M. Sergeeva // Influenza and Other Respiratory Viruses. – 2016. – Vol.5. – P. 247.-253.

# EVALUATION OF A MULTIPLEX TAQMAN REAL-TIME REVERSE TRANSCRIPTION-PCR ASSAYS FOR THE DETECTION OF A( H1N1) PDM INFLUENZA VIRUSES IN CLINICAL SPECIMENS

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The pandemic virus of Influenza A (H1N1)pdm09 (new Californian strains of 2009) became the absolutely new variant of flu virus, which was not previously circulating among humans and to which most people don't have immunity emerges and it could be transmited among humans. The 2009 pandemic virus had been spread globally quikly, and on 11 June 2009, the World Health Organization (WHO) declared the first influenza pandemic since 1968–1969[1]. Genetic analysis of new virus A(H1N1)pdm09 showed that it is 4-fold reassortant and already contains internal segments, that belong to the viruses of human influenza, birds and two separate lines of flu of pigs: the North-American and Eurasian lines[2]. April 2010, laboratory-confirmed infections of pH1N1 influenza virus was identified in 212 countries and overseas territories in April 2010, and fact of more than 18,000 laboratory-confirmed deaths was reported to the WHO worldwide. And in Ukraine have been reported the information of more than 1128 deaths in Ukraine in the same period was reported by Ministry of Health of Ukraine[4].

Research evaluation of virus of Influenza A (H1N1)pdm09 was carried out by Ukrainian scientists and it was detected[3], Ukrainian isolates of influenza virus had a high genetic identity (99%) to the pandemic strains A(H1N1)pdm09, this was observed in the period of 2009-2010 in other countries.

This pandemic A(H1N1)pdm09 virus has been widely circulating across the globe since 2009, and now it is established in human populations as a seasonal influenza virus. But during the epidemic season of 2015-2016 in Ukraine pandemic influenza virus subtypes A(H1N1) pdm09 caused a considerable deaths among the human population of Ukraine. During 40

week it was registered 370 laboratory confirmed deaths caused by influenza, and 81,4% cases were caused by pandemic influenza A (H1N1)pdm09 virus [4].

Therefore modern diagnostics of the Californian strains of virus A(H1N1) pdm09 and monitoring of pandemic strains of virus subtypes A (H1N1) pdm09 across the south and east areas of Ukraine, where the birds influenza viruses A(H5N1) circulate naturally[5] remain actual problem for today.

The goal of our research work was to evaluate a Multiplex TaqMan Real-Time RT-PCR for the rapid and specific diagnostic of pandemic influenza virus A (H1N1) pdm09 in clinical samples of patients with influenza.

We used Human Influenza virus reference strains A/FM1/47 (H1N1), A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1), B/Hong Kong/330/01 were kindly provided for our study by Svitlana L. Rybalko and WHO Collaborating Center for Influenza (CDC, USA) for evaluation the specificity levels. Human respiratory samples (nasopharyngeal swabs and aspirates, sputum) from Viral Influenza (VI) patients (n=10) were kindly provided by Irina G. Kostenko (the Main Military Clinical Hospital of Ukraine). The clinical samples were validated by Seeplex® Influenza A(H1N1pandemic) RT-PCR assay (Seegene Inc., South Korea) and TaqMan Influenza A (H1N1) Assay Sets [10]. The polymerase chain reaction in real time (Real-Time RT-PCR) was done for Applied Biosystems(ABI PRIZM 7000/7500).

It was found that the Multiplex TaqMan Real-Time RT-PCR assay, designed primers and the TaqMan-probes(test kit «DIA Influenza H1N1») identifed the RNA of influenza pandemic California strains of A ( H1N1) pdm09 in clinical samples with 100% sensitivity and specificity.

Ten-fold dilutions of A (H1N1) pdm09 recombinant plasmids pIMC-13(M-gene), pIHC-21(H5-gene) и pINC-20(N1-gene) were used for the determination of detection limits and the amplification efficiency of the assay. Samples were tested in triplicate for each dilution. Therefore, analytical sensitivity of test is 10 copies per reaction.

The proposed TaqMan Real-Time RT-PCR assay is an effective tool for fast and precise detection and diagnostic of the pandemic strains of influenza virus A (H1N1)pdm09.

1. Michael A. Jhung, David Swerdlow, Sonja J. Olsen et al. Epidemiology of 2009 Pandemic Influenza A (H1N1) in the United States//2009 Pandemic H1N1 Epidemiology -2011:52(Suppl 1).-P.13-26.
2. Mironenko A. P., Onishchenko O. V., Golubka O. S., Kurinko N. A., Leibenko L. V. Pandemia of influenza in Ukraine in 2009–2010

winter season: lessons learned for future // Liky Ukrainy.–2010.–N 10 (146).–P. 86–90.
3. Leibenko, L. V., Polischuk V. P., Mironenko A. P. Phylogenetic analysis of nfluenza A(H1N1)pdm viruses isolated in Ukraine during the 2009–2010 pandemic season// Biopolymers and Cell. 2013. Vol. 29. N 2. P. 143–149.
4. Flu and ARVI in Ukraine: Informative bulleten.- Kyiv.- 2016 - P.19.
5. Research and information report. Analysis of distribution of bird flu of H5N1 in the world and Ukraine in 2005-2010.- Simferopol.- 2011.
6. World Health Organization. WHO information for laboratory diagnosis of new influenza A (H1N1) virus in humans. Diagnostic Recommendations H1N1.-2009. http://www.who.int/csr/resources/publications/swineflu/WHO

# ANTIVIRAL DRUG WITH WIDE RANGE OF ACTIONS, THEIR NATURE AND MECHANISM OF ACTION

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For a long time the ideology of creating a medicine was based on the synthesis of compounds that bonded with the active center of the target protein with a high association constant within $10^{-10}$-$10^{-12}$ M. This results in the inhibition of this target protein. Yet the cells quite easily produce resistance to antibiotics, antiviral and anticancer drugs. As a result, they lose their effectiveness. So, it becomes necessary to build a new concept of biologically active substances (BAS) with a wide range of action and perseverance to mentioned resistance. Nowadays the development of natural origin BAS, which have an ability to influence the regulating mechanisms of functions of different organs, tissues and systems with various infectious diseases, is very important. The ribonucleic acids (RNAs) are considered to be the most promising BAS. They can have a wide range of different conformations and carry out a large number of cellular functions due to specific RNA-RNA, RNA-DNA or RNA-protein interactions. Success in the study of these various RNA interactions allowed

developing a number of technologies that have made it possible to create new BAS for treatment of various infectious diseases.

Previously, in our study of the antiviral action mechanism of interferon cycle we found out that synthetic analog of “core” 2'-5'oligoadenilates (2'-5'$A_3$), which have a wide spectrum of antiviral activity. They can bind to regulatory cell’s proteins, change their conformation and functional activity. Association constants for some cytokines, protein kinase and calcium binding proteins at interaction with 2'-5'$A_3$ and its analogues are measured within $10^{-4}$ – $10^{-6}$ M. And the properties study of 2'-5'$A_3$ complexes with these proteins evidences on their impact on conformation, affinity and functional activity of the studied proteins.

A more detailed NMR spectroscopy study of the binding site of 2'-5'$A_3$ with human S100A1 protein in the apo-form showed changes in its conformation due to amino acid residues Thr39, Glu40 and Phe44. An important section of the S100A1 structure, responsible for the interaction with the target ligand is the linker region. It is remains of Glu40 - Val51 loop between two EF-hand motifs that contain an aromatic amino acid Phe44. The obtained changes in Phe44 region basically determine the conformation of the entire loop, confirming the structural changes of the 3D structure of apo-S100A1. This give us opportunity to assume that for 2'-5'$A_3$ and its analogs in the linker region of $Ca^{+2}$ binding proteins there are specific binding sites, which resulting in a change of conformation and functional activity. Probably such binding sites of oligoribonucleotides (ORNs) beyond the active center exist also for other proteins.

Mass spectrometric study of interferon and S100A1 showed their ability to bind up to five 2’-5’-$A_3$ molecules and their analogues. CD spectroscopy revealed trustworthy change in alpha helix of proteins structure by more than 5% at such interaction.

The results obtained during the study of eight different protein kinases showed the ability of 2'-5'A3 and its analogs to influence on their activity by not binding to the active center of enzymes. Herewith activity curve of protein kinase Avrora depending on the concentration of 2'-5'A3 and its "epoxy" analog has W-shaped character. Such character of protein kinase activity indicates on the ability of ORNs to change the conformation of the protein and thus to have both inhibitory and stimulatory activity with respect to its functions.

Thus, the properties study of 2'-5'$A_3$ allowed to confirm the possibility of creating an antiviral drug based on ORNs with a broad spectrum of action. Since chemically synthesized analogues of 2'-5'$A_3$ are too expensive and currently technologically unavailable for industrial production it became necessary to find cheaper natural ORNs. The monitoring of ORNs selected from highly purified total yeast RNA was made. As a result a number of BASs were obtained. Mass spectrometry

study showed the presence of the dominant faction of ORNs consisting of 3 to 6 nucleotides including similar to 2'-5'$A_3$. By means of FT-IR and FT-Raman spectroscopy complexes of ORNs with alcohol sugars that have the maximum ability to change conformation and functional activity of cytokines and $Ca^{+2}$ binding proteins, as previously was shown for 2'-5'$A_3$ analogues, were selected. On experimental models were studied the BAS optimal concentrations and protocols of preventive and therapeutic actions under the commercial name Nuclex against viruses of pandemic influenza, avian influenza, and also against the viruses that cause acute upper respiratory tract infection, including parainfluenza and adenovirus. Also Nuclex's antiviral effect against various types of hepatitis, including hepatitis C as well as herpes group, including cytomegalovirus and Epstein-Barr virus was shown.

The mechanism of antiviral action of Nuclex was studied and it was shown that it affects (like 2'-5'$A_3$) the conformation of cell's regulatory proteins and virus' capsid proteins by changing their structure. In the case of pandemic and bird influenza it inhibits activity of hemagglutinin and neuraminidase in a wide range of low concentrations of both injection and capsule drug forms. Similarly Nuclex affect capsid protein of Epstein-Barr virus. It also affects the conformation and functional activity of the NC protein of human immunodeficiency virus (HIV), inhibiting up to 95% capacity of NC protein of the HIV virus to untwist viral RNA, which stops the process of its further replication.

Following the results of clinical trials the Ministry of Health of Ukraine registered Nuclex as antiviral drug with a wide spectrum of antiviral action and now it's more than 10 years presented on the pharmaceutical market of Ukraine.

Obtained data indicate that RNA is effective and promising BAS. They can be useful for the development of new antiviral, antibacterial and anticancer drugs with a wide spectrum of biological action. Their ability to change conformation and functional activity of target proteins allows us to assume the possibility of creating new drugs to which cells cannot develop resistance that is a priority task of modern science. Presented study protected by USA, the EU, India, China, Russia, Ukraine patents and published in national and foreign scientific journals.

1. Tkachuk Z. Yu. 2'-5'-Oligoadenylates as a «tool» of innate immunity. Biopolymers and Cell. 2013. Vol. 29. N 4. P.1-10.
2. Tkachuk Z. Multiantivirus compound, composition and method for treatment of virus disease. US patent application publication– 2012, Sept.13, № 0232129 A1.

# THE NOVEL ACRIDONE-4-CARBOXYLIC ACID AMIDE INHIBITORS OF INFLUENZA VIRUS REPRODUCTION WITH ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES

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Nowadays a problem of resistance pathogens to many novel drugs has current importance both for medicine and agriculture. It should be noticed that treatment of serious viral infections often is associated with reduced immunity and as a result it is supported by opportunistic bacterial and fungal infections . In regard to this, it is promising to search for agents that would have a complex effect toward different pathogens.

In our previous researches among series of unsubstituted amides of acridone-4-carboxylic acid (ACA) were found compounds that effectively inhibited the reproduction of some strains of influenza A virus (H1N1) and hepatitis C virus. At the same time, it was shown that these agents effectively inhibited RNA synthesis *in vitro* on a model transcription system of phage T7.[1].

Thus, the main aim of our study was in the synthesis and searching potential compounds that could demonstrate a manifold effect against the diversity of pathogenic agents in the midst of new amides of fluoro- and

chloro-substituted ACA. At initial screening some substances of this series demonstrated an antiviral activity against H1N1 influenza A virus in concentration of 70-10 μM. Alongside with that these compounds proved to be reliable inhibitors of RNA synthesis in the last-mentioned transcription system (inhibition concentration in measure of 20-1 μM)

A primary screening of antibacterial and antifungal properties of new amides, that showed antiviral activity, was investigated by the generally accepted disc diffusion method [2]. It was revealed that ACA75, an active inhibitor of H1N1 influenza A virus (EC50 = 10 μM) also showed a pronounced activity towards *Pseudomonas aeruginosa* ATCC 27853 (concentration - 10 micrograms per disc, diameter of inhibition zone 25 mm) with changes in bacterial cell morphology. The antifungal properties of this compound were observed against the yeast-like fungi *Candida albicans* ATCC 10231 (concentration - 10 micrograms per disk, diameter of inhibition zone 16 mm). It should be noticed that ACA75 inhibited RNA synthesis at concentration of 12 μM.

The results prove that synthesized amides of ACA are promising small molecules that can be used for searching new compounds that exhibit various biological activities acting as antiviral, antibacterial and antifungal agents, one of their possible mechanisms may underlie the inhibition of pathogens' RNA synthesis.

1. L. G. Palchykovska, S. L. Rybalko, S. Yu. Rymar, D. B. Starosyla, I. V. Alexeeva, A. D. Shved Use of the model DNA-containing enzyme systems to search for inhibitors of nucleic acid synthesis in viruses and bacteria // Laboratory diagnostic – 2010. – T.53,№3. –C. 31 –37

2. Multicenter Comparison of the ISO Standard 20776-1 and the Serial 2-Fold Dilution Procedures to Dilute Hydrophilic and Hydrophobic Antifungal Agents for Susceptibility Testing / Gomez-Lopez A., Arendrup M. C., Lass-Floerl C. [et al.] // J Clin Microbiol. – 2010. – Vol. 48, № 5. – P. 1918–1920

# THE ABILITY OF ANTIVIRAL RNA-BASED DRUGS CAUSE CONFORMATIONAL CHANGES OF INTERFERON A-2B

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It is known that antiviral RNA-based drug increases interferon production and stimulates non-specific antivirus protection but the molecular mechanism of its action is still unclear. So the aim of our research was to study the ability of total yeast RNA and complex of total yeast RNA with alcohol sugar – D-mannitol [2] to affect conformation and stability of Interferon (IFN) α-2b - key protein of the antiviral cell defense mechanism.

To investigate interaction, conformational changes and stability of IFN protein at the presence and/or absence of the ligands the fluorescence and CD (circular dichroism) spectroscopies were used. All experiments were performed on spectrofluorometer Jasco FP- 8200 and CD spectrometer Jasco J-815 with the peltier temperature cell holder.

Obtained thermal denaturation profiles of IFN α–2b alone and in the presence of RNA and RNA:D-mannitol complex show that addition of these ligands leads to thermal stabilization of protein and could indicate on interaction between IFN α-2b and mentioned compounds that cause the changes of its conformation. For further confirmation of this assumption we calculated dissociation constant that appeared to be relatively weak (micromolar) in both cases. Interestingly, that CD spectroscopy data demonstrate that both RNA and RNA:D-mannitol complex caused tiny alterations in 3D structure reflected in a small decrease of molar ellipticity within both helical bands. The analysis of IFN secondary structure changes

by CDNN software shows that adding of RNA or RNA:D-mannitol complex leads to decreasing of α-spiral components in the protein structure up to 4.1% or 5.5%, respectively, and small increasing of β-turn and Random coil components.

We suppose that RNA and RNA:D-mannitol complex acts as a compounds, altering the secondary structure of the Interferon and in this way change its biological activity. Our results are important for unrevealing molecular mechanism of RNA-based compounds antiviral actions.


1. Антигрипозна активність препарату нуклекс/ З. Ю. Ткачук, С. Л. Рибалко, Л. Д. Жаркова, Д. Б. Старосилаб Л.І. Семернікова// Доповіді Національної академії наук України, 2010, №9, ст. 191-196.
2. Tkachuk Z.Yu. (2013) Pat. US8420617 B2, US 13/046,240. Multi-antivirus compound, composition and method of treatment of virus diseases / Owner name: BIOCELL LABORATORIES, DISTRICT OF COLUMBIA; Effec. 2011.03.10; pub. 2013.04.16.


## THE NEW OXIAMIN SUBSTANCES AND THEIR ANTIVIRAL ACTIVITY


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High prevalence of infectious diseases of viral etiology on the one hand, and a small amount of drugs for their prevention and treatment on the other, stimulate of renovation and expansion currently known range of

antiviral agents. Typically, the creation of drug begins with the search for leader-compound with certain pharmacological properties as structural prototype of medicine.

The aim of our screening was to establish possible correlation between the structure of substances and their antiviral action. According to structure of radicals, new synthesized compounds [1] based on alicyclic and saturated heterocyclic condensered oxyamin systems divided into: adamantanebased (27 substances), bornyl /norbornyl (7 substances), and substances with cyclic and alicyclic radicals in alkoxy group (8 and 11 respectively). Antiviral activity of agents was evaluated against reference-strains of different types of viruses with certain characteristics. The *Influenza virus* type A/FM/1/47(H1N1) with infection titer of allantois culture 7.0 lg EID50/0,2 ml and hemagglutinin titer – 1:512 GAO/0.2 ml. Second test-model was lyophilized *Herpes simplex virus* (HSV) 1-st antigenic type (strain VC). Infectious titer for HSV was 4.5 to 4.25 lg TCD50 /0.1 ml by cytopathic effect (CPE) in cell culture of hamster kidney (BHK). *Bovine viral diarrhea virus* (BVDV) was used as a surrogate of *Hepatitis C virus* (test model VHC). The infectious titer of the virus material with BVDV was 6-7 lg ІД50 held after ten passages in cell culture of kidneys calf Madin Darbi (MDBK). The *Poliovirus* of the first type (vaccine strain) was used as a test model of *Enterovirus*. Its infectious titer was 6 lg TCD50 /0,1 ml by CPE in the cell culture HEp-2.

Evaluation of the antiviral activity of the studied compounds was performed *in vitro* according to standard techniques [2].

The results revealed some correlation between chemical structure of substances and antiviral activity of the investigated compounds. Drugs with alicyclic substitutes in alkoxy group were the most active toward various types of viruses. Among of 11 investigated drugs of this group seven showed antiviral action. Five substances were active against the influenza virus and one of them has higher therapeutic index (TI) than reference drug Rimantadine that was equal to 256 [3]. Compounds from this group of substances were effective inhibitors of herpes virus reproduction and surrogate virus of *Hepatitis C*. One agent showed activity against *Enterovirus* with value of TI 16. It should be noted that one compound from group of substances with alicyclic radicals in alkoxy group effectively inhibited the reproduction of three different types of viruses: BVDV, *Influenza virus* and HSV and had high TI (128, 64 and 64 respectively).

Among of studied compounds with cyclic substituents in alkoxy group, only one was highly active against the *Influenza virus* with TI 256. All bornyl/norbornyl substances were inactive toward studied viruses. Only one of the adamantanebased compounds showed well-pronounced inhibitory effect against *Enterovirus* with TI 16.

Results obtained can be useful for further targeted synthesis of new active antiviral molecules.


1. Prytula P.T., Pupysheva O.V., Korotky Yu.V., Mokhort M.A., Lozinsky M.O. Synthesis and spasmolytic activity of (DI)Hydrochlorides and quaternary salts of some adamantyl-containing derivatives of 1-alkoxy-3-dialkylamino-2-propanol / J. Org. and Pharm. Chemistry. – 2010. – Vol.1(29). – P. 25-29.
2. Porva Yu.I., Rybalko S.L., Dyadyun S.T. et al. Cultivation of hepatitis C virus by transfection method / Laboratory diagnostics. – 2010. – Vol.1(51). – P. 20-23.
3. Patent №98535 from 25.05.2012, Bul.№10. Hydrochlorides 1-alkoxy-3-dialkylamino-2-propanol // Korotky Yu.V., Voloshchuk N.M., Rybalko S.L., Starosyla D.B., Shyrobokov V.P., Losinsky V.O.


# THE ROLE OF RESPIRATORY VIRUSES IN THE ETIOLOGICAL STRUCTURE OF RECURRENT OBSTRUCTIVE BRONCHITIS IN CHILDREN


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The role of respiratory viruses in the etiological structure of recurrent obstructive bronchitis in children. The main pathogens acute respiratory diseases in children are a variety of respiratory viruses, which account for 65 to 95% of acute respiratory infections. Respiratory pathogens are the most common person. [4] Each year, the scale of humanity they cause hundreds of millions of cases of infections. The relationship between

these pathogens and the development of recurrent obstructive bronchitis no one in doubt. [1] At the same time direct mechanism of pathogenesis of certain viruses bronchial obstruction syndrome is not fully elucidated. Yes, there is evidence to indicate that there are differences between different viruses that cause the possibility of recurrent attacks of bronchial obstruction by multilevel changes in the immune system [3].

In particular, it was found that these pathogens can act as triggers that induce bronchial attack in patients with allergic genesis of recurrent obstructive bronchitis. At the same time, the possible impact on the development of bronchial obstruction in patients who have allergic diseases is not fully elucidated. [2] One hypothesis indicates that young children develop acute respiratory viral infection cannot end in a typical recovery intervals, and lengthen, thereby causing the development of chronic inflammation. In order to clarify the prevalence of respiratory viruses and their impact on the pathogenesis of infectious and allergic-associated recurrent obstructive bronchitis a study of the role of agents of virus respiratory infections multpleks - performed using PCR analysis in real time. Carried determining human Respiratory Syncytial virus (- hRSv), human Metapneumovirus - (hMpv 1 - 4 - hPiv), human Coronavirus - (hCov), human Rhinovirus - (hRv), human Adenovirus B, C, E - (hAdv) human Bocavirus -) hBov) in clinical material using the polymerase chain reaction (PCR) with hybrydyzatsiyno - fluorescence detection. The study was conducted on 1-2 days of onset. There were examined 55 children which were manifestations of bronchial obstruction syndrome, of which 32 patients were identified infection-associated variant of course recurrent obstructive bronchitis and other 23 patients occurred option allergic disease.

It was found that the development of bronchial obstruction syndrome in children in both groups was associated with acute rhinovirus infection. The study was conducted in spring 2016. The other potential pathogens identified were not. These results indicate intense circulation of rhinoviruses in the population during the study.


11. Nelson textbook of pediatrics, twentieth edition 2016 // Elsevier . – P. 3466-3473
12. Beydon N., Davis S.D., Lombardi E. Et all. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J. Respir. Crit. Care Med. – 2007. – 175 (12) – P. 1304-1345.
13. Plusa T., Ochwat A. Fenspiride in patients with acute bronchitis // Pol. Merkur Lekarski. – 2005. – V.19 (109). – P. 32-36.

14. General and Clinical Pathophysiology / Edited by A.V. Kubyshkin. – Vinnytsia. Nova Knyha Pablishers. . – 2011 – 656. P. 634 – 637.

# THE KEY QUESTIONS OF THE LOGIC OF VIRUS-CELL INTERACTION AND NEW APPROACHES TO ITS CORRECTION

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The persistence of viruses in the organism and their key role in the evolution [1] are doubtless facts. From this point of view, the term "virome" would logically combine micro- and macro- biome into a whole. This approach demands more deliberate usage of the substances that are thought as strictly antiviral if they influence the process of virus-cell interaction. Generally speaking, the approach to the virus infection therapy that tries to replicate the logic of antibacterial therapy is conceptually wrong, as it ignores the fact that the program of virus development is a cascade of mutual reactions of the virus and the cell. Without the "correct" reaction of the cell, it is impossible for the virus to develop.

It is usual for today's therapy to use highly specific antiviral medicines. The problem is that this approach not only stimulates the selection of the pathogen but also destroys the intimate, stable relations between other viruses that persist in the organism [2, 3]. The consequence of this tactics is the decrease of the medicines' effectiveness and the increase of the virus pathogenicity.

The usage of the substances that change the logic of the cell's response to the viral infection allows correction of the development of the infection. Among such substances, we studied low molecular compounds that stimulate innate antiviral immunity: tilorone structural analogs, muramyl dipeptide (MDP) derivatives, and phenolic compounds.

In 2003 B. Rzigalinski, S. Patil, and S. Seal laid the foundations for a radical nanomedicine [4, 5]. Its object is ceria nanoparticles that have antioxidant and regenerative properties. In our works, we were showed that

in the circumstances of the viral infection nanoceria provides trigger-hysteresis properties and change the direction of the development of the acute infections.
The complex approach that we developed allows achieving significant success in the antiviral therapy without directly affecting the pathogen.


1. Ryan F. 2009. Virolution.
2. Duerkop B.A., Hooper L.V. 2013. Resident viruses and their interactions with the immune system. Nature Immunology, 14(7), 654-659.
3. Cadwell K. 2015. The Virome in host health and disease. Immunity, 42, 806-813.
4. Rzigalinski B. et al. 2003. Cerium oxide nanoparticles extend cell longevity and act as free radical scavengers. US Patent.
5. Seal S. et al. 2013. Inhibition of reactive oxygen species and protection of mammalian cells. US Patent.


## COMPARATIVE CHARACTERISTICS OF INFECTIOUS METHOD OF DETERMINATION OF ADENOVIRUS TITER IN CELL CULTURE


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Adenovirus infection is a widespread human disease which children are most sensitive to 4 years. During the year the infection has a constant frequency and distribution of up to 8% of all viral diseases. Among respiratory viruses adenoviral infection takes the second place after flu. To identify the pathogenic agent among many factors the method of determining the titer of infectious virus in cell culture is commonly used. The aim of our study was to conduct a comparative characterization of known methods of adenovirus titration and determine the best way for

further work. The cell cultures Hep-2 (human laryngeal carcinoma), A549 (human lung carcinoma), MDBK (bovine kidney), and reference strain of human adenovirus serotype 5 were used. Infectious virus titers were determined: using the virus CPE by endpoint dilution according to Reed-Muench method; using cytomorphological method with the acridine orange (AO) dye; by the method of using an agar coating for vital titration by plaque formation; colorimetric method using MTT and resazurin. Using the titration method of the virus by CPE for breeding material endpoint the adenovirus titer was defined as $4*10^3$ CPE units/ml. The method is simple and less costly, but it is not very sensitive, subjective, and difficult in assessing the results. Adenovirus titer determined by cytomorphological method using the AO was equal to $7*10^7$ ICU/ml. The method is more sensitive. Using fluorescent dye and AO microscopy, specific intra-nuclear inclusions formed in the accumulation of viral progeny in a cell can be detected after 48 hours. Disadvantages of the method are the presence of specially trained staff, fluorescent microscope, and a carcinogen dye.

The adenoviral titer determined by the method of using an agar coating for vital titration by plaque formation was equal to $4*10^8$ PFU/ml. The method is still considered as the most sensitive and specific. However, it requires the use of cells monolayer for long-term cultivation, and requires significant amounts of cost for reagents. Using the colorimetric method based on MTT (3-(4,5 dymetylthriazol-2-yl)-2,5-dypheniltetrozolium bromide) serotype 5 adenovirus titer was determined. It was equal to $3,2*10^8$ CPE units/ml. This method is highly sensitive, automated, simple in conducting, and fast. Perhaps the only drawback of this method is the need for a specific dye and counting device for the optical density of the cells. The specific dye MTT in our studies was replaced by resazurin solution (25 mg/ ml), which significantly reduces the cost of research, but keeps highly sensitive colorimetric analysis. Infectious titer adenovirus determined using resazurin solution was $2,4 * 10^8$ CPE units/ ml. Thus, the same viral material was titrated using the above methods revealed the advantages and disadvantages of each method.

| Method of investigation | Viral titer | Days | Sensitivity | Drawbacks of method |
|---|---|---|---|---|
| by plaque formation with agar | $4*10^8$ PFU/ml | 7 | high | Use of cells monolayer for long-term cultivation, costly reagents |
| by CPE for breeding material endpoint | $4*10^3$ CPE units/ml | 7 | low | Subjective results |

| | | | | |
|---|---|---|---|---|
| Cytomorphological method | $7*10^7$ ICU/ml | 2 | high | Highly qualified researcher, fluorescent microscope, carcinogenic AO |
| Colorimetric method based on MTT | $3,2*10^8$ CPE units/ml | 3-5 | high | The high cost of MTT |
| Colorimetric method based on resazurin | $2,4*10^8$ CPE units/ml | 3-5 | high | no weaknesses |

The method of fluorescent focuses described in literature is fast as cytomorphological method using AO. However, it requires highly skilled researcher, a large number of costly reagents, and preliminary optimization study terms, and has a subjective component in evaluating the results.

So, after analyzing all the methods for establishing adenovirus titer in cell culture, their sensitivity, required reagents, and the duration of the analysis it can be concluded that the colorimetric method with the use of resazurin solution is the best approach.

# SESSION "PLANT VIRUSES”

# VIRAL DISEASES OF CEREALS CROPS IN ORGANIC FARMING

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The term "organic agriculture" brings together all the systems of agriculture that are engaged in agricultural production using methods that are safe for the environment. Certified organic producers adhere to the principles of the world are embedded in the local economy, geoclimatic and cultural environment. Organic agriculture is a healthy farmer, products and the environment. Methods used taking into account use the natural qualities of plants, animals and landscape. Manufacturers of organic products are looking for ways to give up practices that threaten life, depleting resources and polluting the air and water. Basically, organic system considers soil fertility as a key to successful production. So the health and life of the soil remain one of the main objects of attention of organic Agriculture Technology improving soil fertility balance physical, chemical and biological characteristics of the soil, thanks to the methods such as crop rotation, usage of herbs for cattle, use of natural fertilizers recycling waste from plants and animals, cultivation of land and injection the necessary minerals from time to time [1].

Organic products increase popularity in Ukraine and the world. The leader of the organic agricultural enterprises in Ukraine is "Agroecology" in the Shishatskiy district of Poltava region. This company is certified in accordance with the requirements of the EU Directive № 834/2007, which provides state regulation of organic products in the countries members of the European Union and led to the demand for its products in Ukraine and in many European countries [2, 3]. In the fields of the enterprise "Agroecology" for nearly 40 years do not use poisons and chemical fertilizers. Use of organic fertilizer (humus, green manure, etc.) provided annual increase of humus content on 0.5% and is now at an average of 5.2% (!). For comparison, Poltava soils currently have humus content about 2.6 ... 3.6%.

In Ukraine, a significant threat to cereals from plant viruses particular *Wheat streak mosaic virus* (WSMV) and *Barley yellow dwarf virus* (BYDV) [4]. *Triticum Mosaic Virus* (TMV), *Brome Mosaic Virus* (BMV) were revealed much less, and *Barley stripe mosaic virus* (BSMV) was observed sporadically. The most studied is WSMV, it is easy to study because it has specific symptoms and laboratory experiments are well transmitted mechanically. Symptoms of WSMV (genus *Tritimovirus*, family *Potyviridae*) is difficult to confuse with other diseases, which is characterized in autumn are light green chlorotic spots, mosaic spring and appears as light green and yellow strips of different lengths, which extend parallel to a vein. Symptoms of VYDB lesions (redness at the top of the sheet) are often similar to the influence of other abiotic factors: temperature changes, man-made pollution violations farming and so on. Recommendations to protect winter wheat and other cereals that are grown by intensive techniques are developed [4]. We study biological plant protection method [5]. We begin investigations of viral diseases of cereals under conditions of organic farming [6].

Inspection of grain crops to defeat viral diseases in the conditions of organic farming in 2009 and 2014-2016 showed that winter wheat crops of different varieties (Rozkishna, Kalita, Vidrada, Vilshany, Smuglanka), barley Astoria and oats Neptune by virological methods (ELISA "sandwich option" ELISA), reverse polymerase chain reaction (RT-PCR) and transmission electron microscopy in 2014 was found wheat streak mosaic virus (WSMV) [6]. In 2016 for grain crops was not any of circulating and prevalent in Ukraine and the world WSMV, BYDV and others. Season 2015-2016 was not favorable for the carriers of viral diseases through late autumn stairs of winter wheat and a large amount of spring rainfall. In some years, only a few diseases of oats by BYDV, but it were well below the economic threshold of harmfulness (ETH). It is due to the low number of vectors of BYDV and WSMV - aphids and mites *Aceria tritici* Schev., Provided that organic farming (balance entomofauna); create natural conditions for accumulation of entomophages. After determining factor in the efficient and safe use of natural populations entomophages akaryphages, entomopatophages and enter test their effectiveness - of the ratio of predator-prey or percentage of affected individuals pest pathogen [2, 5]. Particular importance in organic agriculture plays cultivation of the soil, which provides improved soil structure and provides power supply for soil microorganisms. A survey of winter wheat farms in Poltava and Mykolaiv regions showed that lesions caused by WSMV of Smuglanka, Antonovka varieties, and was more than 60% in liquefied and weeded fields.

In Tetiivskyi district of Kyiv region on crops with high intense of industrial technology in combination with biological (organic) farming

revealed a slight (<2%) lesions by WSMV of wheat plants of only one variety Yasochka, that is significantly below the economic threshold of harmfulness.

Our studies suggest that under prolonged maintenance of the system of organic farming, reduced the threat of damage to the crop cereals staggered through viral diseases. Experience of enterprise "Agroecology" indicates that organic production can become the priority of the state agricultural policy, which will allow Ukraine to occupy a leading position in the market of agricultural products within country and Europe.


1. Organic production and the European Union / European Parliamentary Research Service / http://www.europarl.europa.eu/RegData/etudes/IDAN/2015/548989/EPRS_IDA(2015)548989_EN.pdf
2. Antonets S.S., Antonets A.S., Pisarenko V.N. etc. Organic farming: the experience of Private Enterprise “Agroecology” Shishatsky district Poltava region. – Poltava: Publishing center PDAA. – 2010. – 200 p.
3. Antonets S.S., Antonets A.S., Lukyanenko G.V., Pisarenko V.M. Social and ethical principles of organic farming // Herald of Poltava State Agrarian Academy – 2013. – # 2. – P. 7-9.
4. Mishchenko L.T. Viral Diseases of Winter Wheat. – Kyiv : Fitosotsiotsentr, 2009, – 352 p.
5. Korniychuk M.S., Vinnychuk T.S., Parminska L.M. Protection of crops from pests and diseases by organic production technologies // Proceedings of NSC "Institute of Agriculture NAAS." –2014, # 1-2. – P. 98-110.
6. Mishchenko L.T. Wheat Streak Mosaic Virus in crops when grown on different technologies // Proceedings of the All-Ukrainian scientific and practical. Conf. dedicated to the 100th anniversary Birthday. Acad. VF Peresypkin "Phytopathology: Present and Future" 16-18 October 2014 Nat. University of Life and Environmental Sciences of Ukraine / – K.: Publishing center of NUBiP Ukraine. – 2014. – P. 29-30.

# TOBACCO MOSAIC VIRUS INTERACTION WITH LIPOSOMAL SUPRAMOLECULAR STRUCTURES BASED ON THE ANTIVIRAL ACTIVE NANOMATERIALS

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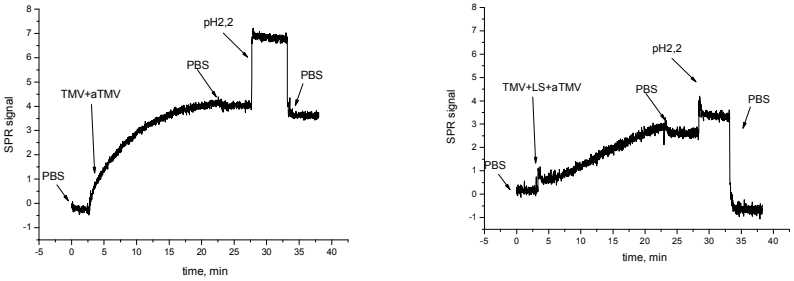
The development of efficient antiviral drugs is one of the topical problems in modern virology. But their safe and address transport to the appropriate targets is very important as well. One of the approaches to the solution of this problem is the forming of the lyposomal supramolecular structures (SMS) based on the antiviral active nanomaterials [1]. Earlier we reported the SMS designed using bionanomaterials, namely, glycans, glycolipids and thiosulfonates and demonstrated their high activity against the Tobacco Mosaic Virus (TMV) [2]. The aim of the present work was to demonstrate the influence of the mentioned SMS on the TMV functionality.

For the investigation of the impact of the SMS on the TMV binding properties the interaction between specific antibodies and virus was used as a model reaction. The reaction was investigated by the surface plasmon resonance method using a scanning SPR spectrometer "BioHelper-01" designed in the V.Ye. Lashkaryov Institute of Semiconductor Physics, NAS of Ukraine. Since the lyposomes themselves do not cause the reasonable change of the signal, the preincubation of the reaction mix components (namely, virus, antibodies and lyposomes) with further comparison of the signal level from the mix with and without lyposomes was applied. For the correct immobilization of the complex, the pretreatment of the sensor surface by the protein A *Staphylococcus aureus* was used (not shown at the graphs).




a b

Fig.1. Immobilization of the preincubated complex of TMV and specific antibodies in the absence (a) and in the presence (b) of the glycan-containing lyposomes

As it is demonstrated at the Fig.1 the incubation of the untreated virus (Fig.1a) and the virus, preincubated with above mentioned nanocomposite (Fig.1b), with antibodies allows one to state the complexation with the nano-composite changes the binding capabilities of the virus which make it potentially useful as virus inhibitor.

1. Kovalenko O.G. Principles and methodology of the creation of agents for sanitation and protection of plants from the viral infections based on bionanomaterials // XIII SMU Congress Yalta, 2013. –P.455.
2. Vasiliev V.N., Kovalenko O.G., Karpenko E.V. Application of surface-active rhamnolipids for formation of the supramolecular structures with antiviral activity // “Nanotechnology and Nanomaterials” (NANO-2013). 25 August 1 September 2013, Bukovel. Ukraine. Book Abstr. – 2013. – P. 266.

# USE OF POTATOES COLLECTIONS FOR DETECTION OF VIRUS RESISTANCE SOURCES

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Food and Agriculture Organization (FAO) indicate potato one of the main factors of food safety of the international community [1]. Ukraine occupies a leading place in the world in production of potatoes. Almost half of the potato acreage is concentrated in the steppe zone of Ukraine.

Ustymivka Experimental Station of Crop Production is a basic research of the National Centre for Plant Genetic Resources of Ukraine. The quantity of the station concentrated gene pool of plants exceeds 28 thousand samples of 133 crops, including potatoes – 640 samples. Collection of various crops and their wild relation, which are supported by recognized national heritage, which have no analogues in Ukraine and can be used in breeding, research and training programs [2].

Maintaining a collection of potatoes samples in the state of viability and genetic authenticity ensures efficient use of national heritage. Biological feature of potatoes - vegetative propagation method usually provides safety heterosis effect for a long time. Sustainability genotypes supported by their annual planting in the field.

Research viral diseases of potatoes are critical because viruses spread simultaneously with planting material and lead to yield losses of about 70 %. Control of viral infections in plantations of crops using their natural resistance to pathogens – important task for selection as a perfect solution to the problem of plant protection and ecological safety of the environment. So finding sources of resistance to viral infections should be given special attention [3].

The research were performed in the laboratory of technical, forage and vegetable crops Ustymivka Experimental Station of Plant Production

Institute of V.Ya. Jurjev of National Academy of Agrarian Sciences of Ukraine in 2013-2015. The source material in our research were samples of potato collection in number 640 varieties of domestic and foreign selection. Methods of studying common [4].

Our aim was the selection of varieties of potatoes, which in the southern steppes of Ukraine are characterized by resistance to curl leaf disease- causative agent L virus (Potato leaf roll virus, PLRV); wrinkled mosaic virus – causative agent Potato virus Y in various combinations of the virus Potato virus X, Potato virus S, Potato virus A, Potato virus M (rarely seen a virus-PVY).

The study material was conducted under natural infectious background for the 9-point scale resistance. As control were used virus resistant varieties: Apta, Shwalbe, Agwila, Karla (Germany). The results of Phytopathological evaluation at the level of standards (8-9 points) selected white varieties resistant to wrinkled mosaic: Horlytsya, Rum`yanka, Fakel, Posvit (Ukraine), Dorisa, Omega, Grata (Germany), Aphrodite (Netherlands) early Bryanska rannya (Russia) and others. As the virus resistant to leaf curl distinguished varieties: Vityaz, Posvit, Dymok (Ukraine); Bintje, Sante, Desiree, Jaerla, Mansour (Netherlands); Dorisa, Turbella, Alpha, Tempora, (Germany); Svitlyachok (Moldova); Eta (Czech Republic); Zhukovska (Russia) and others. In assessing the resistance of varieties to leafroll mosaic vius were distinguished such varieties: Borodyans`ka rozheva, Rum'yanka, Drevlyanka, Vityaz (Ukraine); Aphrodite, Desiree (Netherlands) Runo (Germany), Krystal (Russia).

The potato samples were tested for latent viruses contents. Using electron microscopy, ELISA and RT-PCR was confirmed visual observations and were distinguished varieties with high field virus resistance to pathogens of viral diseases that can be recommended to practical breeding use.


1. FAO Statistical Yeabook, 2014. Europe and Central Asia Food and Agriculture. Food and Agriculture Organization of the Unitid Nations. Regional Offise for Europe and Centrel Asia [Text]. – Budapest, 2014. – 130 p.
2. Bondus RO The rate of reaction potato varieties for cultivation in the southern steppes of Ukraine: Author. Dis. candidate. Agricultural Sciences: 06.01.05 / RO Bondus; Acting Eng. Acad. Agrar. Science. Inst Ya. VJ St George. - Kharkov: [b. in.], 2009.- 18 p.
3. Viral infection of potatoes and of course the conditions of simulated microgravity / LT Mishchenko, V. Polishchuk AP Taran, AI Hordyeychyk.- K .: Fitosotsiotsentr, 2011. - 144 p.

4. Guidelines on research and potatoes [Text] / Institute of Potato UAAS. - Nemishaevo [b. in.], 2002. - 183 p.

# PATHOLOGY OF VARIOUS LEVELS OF COMPLEXITY IN BASIDIOMYCETES UNDER MIXED INFECTION AND ENVIRONMENTAL FACTORS

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The work presents results of many years researches of basidiomycetes fungi quality when grown in natural biocenoses and varying complexity of biotechnological processes. Mycelium and fruit bodies of fungi were analyzed for destruction them with viruses, bacteria, microscopic fungi of various taxonomic groups.

The main purpose of research is a selection of quality mushrooms of different types for forming on their base of chemical fractions - stimulants of plant growth and development («BOA», «Bioecofungi-1»). Screening of natural biomes, analysis of mushrooms of biotech companies, rapid method for diagnosis of pathogens (Boyko O.A., Shevchenko T.P., Boyko A.A., 2013), ELISA, Uhterloni, culture media, the method of determining the growth factor of mycelium (GF) (Bukhalo A.S., 1983) were used for these purposes.

When performing model experiments with GF determination it is worth noting that it was on the basis of incubation the colonies of *Totiviridae* and *Tobamovirus* mushrooms. Under these conditions, incubation of mycelium by viruses caused a significant inhibition of it: uneven growth, a sharp change in color, looseness. Similar pathological processes were also caused by *Pseudomonas fluorescens* (tolaasii) bacteria, *Micogone perniciosa*, *Trichoderma viride*, *Cladobotryum dendroides* fungi and other pathogens of mushrooms. It should be noted that the rate of an infectious process in fungi is often well observed at the visual assessment of the fruiting bodies of mushrooms and morphological features of basidiospore that were studied at different magnifications of microscope. About 40 species of basidiomycetes fungi, which often have a latent viral

infection, but did not meet the quality requirements of mushrooms by the GF indicator, were tested to create stimulants of plant growth and development.

Methodical fragments based on selecting of Glycosidase of microbial cells (Varbanets L.D., Borzova N.V., 2010), and also evaluation of assessment of designed stimulants composition based on the received other methods have been used to generate biological products based on the mushrooms components.

## NEW PROMISING INTRODUCENT YAKON AND ITS PATHOGENS OF VIRAL DISEASES

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One of the most promising crop for the prevention and treatment of diabetes considered yakon (*Polymnia sonchifolia* Poepp. & Endl), which introduced and put into culture in many countries. Due to the content of chlorogenic, coffee acids and other phenolic compounds leaves of yakon have antioxidant properties [1, 2]. Root tubers of yakon contain inulin - a polysaccharide that is easily absorbed by the body and serves as a substitute for sugar in the diets of diabetic patients. As a result of studying the chemical characteristics of the plants recommended yakon in the form of flour, syrup and extract of root tubers and leaves - for cooking infusions of hypoglycemic effect. Protein of root tubers of yakon by the content of essential amino acids far exceeds the protein of wheat, corn and soybeans, it is promising for use as a component of animal feed and as a bioenergetic culture.

Lachman, J. conducted studies showed that the content of important biologically active compounds and chemical composition of yakon grown under different conditions vary quite strongly [1]. Our

research began in the 2009 study of introduction *Polymnia sonchifolia* Poepp. & Endl allowed choosing the optimal soil and climatic conditions for growing this promising exotic species in Ukraine [3]. We improved method of obtaining planting material and agricultural technologies of yakon cultivation using drip irrigation, which contributed to a better accumulation in plants of some important macro and trace elements, including selenium [4]. But in recent years (2013 and 2015), we observed the morphological changes of root tubers destructive significantly smaller and deterioration of their quality [2]. The method of transmission electron microscopy in 2015 we discovered filamentous virions by the length of about 600 nm. This is the first message about defeat in Ukraine of root tubers.

Earlier we saw yakon plants with symptoms of mosaic leaf plates, which by transmission electron microscopy was discovered filamentous by virus particles [5]. There are several studies that have been devoted to the study of viruses that affects yakon. Kuroda and Ishihara [6] found in the field of plants infected cucumber mosaic virus (CMV). But viruses potato leaves curl and Y-, S-, M- potato viruses are not detected.

Testing of our samples of leaves during the growing season of yakon performed by ELISA using antisera to spread in Ukraine X-, Y-, M-, S-potato viruses and somewhat similar in morphology. Founded no serological relationship of these antigens, which is consistent with the results [6].

So we found in some years disease of yakon plants (leaves and root tubers) viral diseases that deserves attention for further research.


7. Lachman J., Fernandez E.C. and Orsak M. Yacon [*Smallanthus sonchifolia* (Poepp. & Endl.) H. Robinson] chemical composition and use – a review. Plant, Soil and Environment, – 2003.– 49, (6): – P. 283-290.
8. Gins M.S., Gins V.K., Kononkov P.F., Dunich A.A., Dashchenko A.V., Mishchenko L.T. Phenolic compounds and fructosans in yacon (*Polymnia sonchifolia* Poepp. & Endl.) cultivar introduced in Ukraine, and in other *Asteraceae* plants as influenced by growth conditions, viral and phytophage injury // *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2015, Vol. 50, № 5, pp. 628-636.
9. Mishchenko L.T., Dunich A.A., Dashchenko A.V. Lyashuk N.I., Janiszewska G.S., Yakon: technology of growing, harvesting and storage of planting material (*Polimnia sonchifolia* Poepp. & Endl.) // Scientific guidelines. - K.: Publishing and Printing Center "Kyiv University", 2012. - 27 p.
10. Daschenko A.V., Novozhylov V.V., Hluschenko L.A., Taran N.Y., Marchyshyn S.M., Mischenko L.T. New promising alien species of

yacon (*Polymnia sonchifolia* Poepp. & Endl.) for medicinal plant cultivation in Ukraine //Agroecological Journal. . – 2016. – № 2. – P. 39-46.
11. Dashchenko A., Dunich A., Mishchenko L. First report about viral infection of yacon plants in Ukraine // 8th Balkan Congress of microbiology, October 2-5, 2013. Veliko Tarnovo, Bulgaria. – P. 110.
12. Kuroda S., Ishihara J. Field growth characteristics of plantlets propagated *in vitro* and line selection for increased percentage of sugar in tuberous roots of yacon, *Polymnia sonchifolia* // Bull. Shikoku Nat. Agric. Exp. Sta. – 1993. – № 57. – P. 111-121.

# INFLUENCE OF SIMULATED MICROGRAVITY ON PLANT RESISTANCE OF SOYBEAN TO SOYBEAN MOSAIC VIRUS

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In Ukraine, the Soybean (*Glycine max* (L.) Merr.) are grown for animal feed, for use in food and industrial purposes. Exports of soy, our country ranks sixth in the world. In the State Register of plant varieties included over 150 different varieties of soybean breeding establishments. Soy bean has a rare combination oil content and protein content with valuable vitamins, mineral and biologically active compounds, which makes soy strategic culture of the XXI century. The advanced countries, using new technologies isolation and purification of proteins made from soybean variety of food: an isolated protein, textured protein, soy sauce, soy milk, lecithin. Ukraine also built up production of soybean products.

Simultaneously soybeans significantly improves soil fertility, so putting Early winter wheat after soybeans rules allow reduction of nitrogen fertilizer several times.

However, to ensure obtaining high yields of soybeans required and high-quality seeds. Not primarily affected by various phytopathogens, including viruses. Research [1-3] found that soy in our country most striking *Soybean mosaic virus* (SMV), *Bean yellow mosaic virus* (BYMV) and *Alfalfa mosaic virus* (AMV). In our laboratory and field studies revealed seed infection mosaic virus soybean varieties Kuban, Cordoba, Kano, Terek. Seed infection manifested only 1,5...2,0 % of plants. If the field vectors of viral infections and low virus resistance some varieties even a small number of affected plants can cause infection of virus that significantly reduce crop and worsen its quality [4, 5]. Study of microgravity on plant growth shows that prolonged exposure tomato seed varieties peace on the international space station "Mir" lead to physiological and biochemical changes in plants tomatoes when grown under field conditions Kiev and Poltava [6].

The results also indicate about changes in plant resistance to phytoviruses resulting aftereffect influence of space flight factors on seeds *Lycopersicon esculentum* Mill. Earlier [7] found that modeled microgravity led to the elimination of the virus striped mosaic of wheat when grown in clinostat "Cycle 2". It was therefore decided to investigate the effect of preplant clinorotation soybean seed for the manifestation of a viral infection in their further cultivation of natural infectious background. Our research 2015-2016 years showed that after preplant clinorotation soybean seeds for 140 days in clinostat "CG-8" in the field of Kyiv and Poltava regions were found staggered ladder plant soybean mosaic virus (SMV). It was found only about 1% of the soybean plant varieties Kano, which had distinct symptoms SMV (Fig. 1, a). In plants of soybean varieties Cano noted specific symptoms soybean mosaic virus, rugosity leaf surface, dark green leaf between the veins swelling. Similar symptoms described previously [8]. Monitoring staggered sowing soybean in 2016 (June, Kiev region.) found a lesion grade Kuban plant seed because of a viral infection. We observed bubble symptoms and swelling dark green between the veins, starting with the first lower real (non cotyledon!) Soybean leaves. The presence of the virus in these plants was confirmed by transmission electron microscopy. We found threadlike virus particles size of 650-750 nm. Revealed viral particles identified by ELISA us as soybean mosaic virus (SMV).

Note that symptoms on plants varieties Kano (option number 17 – before sowing seeds were stored motionless) manifested June 23 and July 9, 2016. In the third decade of July symptoms of viral infection on the leaves of plants trigeminal new real control has not been found. In clinostated

plants from seeds was found two plants with stringent (strict) SMV symptoms, similar to those we observed in another experiment in 2015.

In plants from clinostated seeds were found two plants with stringent (strict) SMV symptoms, similar to those we observed in another experiment in 2015. Such a course of growth for symptoms of SMV can testify about the impact on the viral infection difficult climatic conditions in 2015-2016 years. The drought, waterlogging, temperature weakened immune status of plants. It is possible that infection of clinostated plants occurred through vector – aphids on a background of adverse weather conditions. Therefore, we conducted the first study showed that the method of clinorotation of soybean seeds, even at rest may be promising and deserves further study.


1. Sherepitko D.V., Budzanivska I.G., Polischuk V.P., Boyko A.L. Sequencing and phylogenetic analysis of Soybean mosaic virus isolated in Ukraine. Biopolymers and Cell. 2011; 27(6):472–479.
2. Kyrychenko A.M., Kraeva G.V., Kovalenko O.G. Biological characteristic and identification of soybean virus isolated from different Ukraine regions // Мікробіологічний журнал. 2012; 74(1): – P. 46-50.
3. Sherepitko DV. Molecular-genetic and biological properties of viruses (soybean mosaic potyvirus, alfalfa mosaic alfamovirus) identified on soybean under environmental conditions of Forest-steppe zone of Ukraine. Thesis PhD. – Kyiv; 2012. Ukrainian.
4. Wang A. Soybean mosaic virus: research progress and future perspectives. Proceedings of World Soybean Research Conference VIII (www.wsrc2009.cn), 2009; Beijing, China.
5. Cui X., Chen X., Wang A. Detection, Understanding and Control of Soybean Mosaic Virus // Soybean - Molecular Aspects of Breeding, [eds. A. Sudaric]. 2011. – P. 335-354.
6. Mishchenko L.T. Impact of a real microgravity on the productivity of tomato plants and resistance to viruses / L.T.Mishchenko, A.A.Dunich, O.I. Danilova / Proc. ‘Life in Space for Life on Earth’, 18-22 June 2012, Aberdeen, UK (ESA SP-706, February 2013).
7. Mishchenko L.T. Viral Diseases of Winter Wheat. – Kyiv : Fitosotsiotsentr, 2009, – 352 p.
8. Tryhubenko V., Dunich A., Molchanets O., Mishchenko L. Viral disease of soybean in some regions of Ukraine // Proceedings of the XI International Conference of Young Scientists "Shevchenko Spring" in 2016, – P. 195-197.

# ANTIVIRAL AND PLANT GROWTH-STIMULATING ACTIVITY OF BIOLOGICAL PREPARATION "BIOEKOFUNGI-1"

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Improving the yield and quality of crop production associated with the use of modern plant growth regulators. Growth regulators are natural or synthetic compounds which in low concentrations can lead to significant changes in the growth and development of plants. In recent times they are becoming essential elements of intensive technologies of cultivation of agricultural crops [1, 2]. One of the most important and valuable crops in Ukraine is buckwheat. This culture is grown in different regions of Ukraine, but it is known that buckwheat is affected by pathogens of different taxonomic groups, in particular viruses, so the search for natural product with antiviral and at the same time growth-stimulating properties is an important challenge for modern agriculture [3].

Studies shown, that to prevent disease and stimulate growth and development of buckwheat plants it is perspective to use biochemical drug "Bioekofungi-1", which is based on the components of Basidiomycetes mushrooms and their carriers from higher plants that have been used in the development of the drug. "Bioekofungi-1" was developed by scientists of National University of life and environmental sciences of Ukraine, department of physiology, biochemistry of plants and bioenergetics. It is important to note a comprehensive action of "Bioekofungi-1", which stimulates the growth and development of buckwheat plants and reduces the aggressiveness of pathogens of different nature [4, 5].

We first discovered that "Bioekofungi-1" affects reproduction of

phytoviruses that affect the buckwheat in different agrocenoses. For example, the formation of intracellular inclusions, subject to the defeat of plants of tobacco mosaic virus and buckwheat burn virus, significantly blocked and becomes less than that observed during the cells study in light and fluorescent microscopy.

We first identified that the drug "Bioekofungi-1" increases the percentage of buckwheat seeds germinating to 30-45% depending on the dosage and also affect resistance of adult plants to pathogens of different taxonomic groups. So, on cultivated plots, where seeds were pre-treated with a 0.1% of preparation, it was 83 % of healthy plants, whereas in control plots it was only 23% of unaffected plants.

In the variants of investigations the crystal formation often possess a loose structure while maintaining the characteristics of the cell nucleus, which is extremely important for the growth and development of plants, control of seed infection in laboratory and vegetation experiments. Moreover, these methodological approaches provide an opportunity to identify circulation of tobacco mosaic virus among the accompanying plants (weeds) that surrounds buckwheat in agrocenosis in forest-Steppe and Polesie.

"Bioekofungi-1"preparation showed high efficient in field experiences as a drug with a protective antiviral effect and ability to stimulate the growth and development of buckwheat plants, so it can be used in practice in different agrocenoses of Ukraine.


1. Funatsuki, H., Maruyama-Funatsuki, W., Fujino, K., Agatsuma, M. Ripening habit of buckwheat // Crop science. – 2000. – T. 40. – №. 4. – P. 1103-1108.
2. Deeva V.P., Sheleg Z.I., Kanko N.V. Selective effect of chemical plant growth regulators. – Minsk, "Nauka", – 1988. – 256 p.
3. Shevchuk V.K., Demchenko O.A., Yuzvenko L.V., Didenko L.F., Spivak M.Ya., Gigolashvili A.I., Petrishe O.I. Buckwheat diseases: ecology, properties of pathogens, prevention. – Kyiv. – 2012. – 158 p.
4. Shevchuk V.K., Demchenko, O.A., Yuzvenko L.V. Assessment of the sensitivity of the Fagopyrum tataricum Gaertn collection of different ecological origin to the buckwheat burn virus // Scientific bulletin of Uzhgorod university (Ser. biol.), – №30, – 2011. – P. 161-163.
5. Demchenko O.A., Shevchuk, V.K., Yuzvenko L.V., Spivak M.Ya., Pasechnik L.A. Zhitkevich N.V. Savenko O.A., Boyko O.A., Melnichuk T.M., Shcatula Yu.M., Kirilenko L.V. Monitoring of buckwheat plant resistance to diseases and their prevention in different environmental conditions. Methodical recommendations. – Kyiv: "Fitosotsiocenter". – 2014. – 22 p.

# CONTROL OF PHYTOPATHOGENIC VIRUSES AT THE POTATO FIELDS IN CHERNIHIV REGION AGROCENOSES

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Among numeral illnesses of potato viral diseases have general distribution with the tendency of increase of their harmfulness in the basic regions of growing of potato on many sorts, that use in agricultural business [1]. In the wild Ukraine purchased wide distribution of M-virus of potato (*Potato virus M*), *Potato virus Y* (PVY), *Potato virus S* (PVS), *Potato virus X* (PVX), *Potato leafroll virus* (PVL). Enriching of population is set by new for a region harmful viruses and their strains. Along from ordinary, the necrotizing strains of Y get all greater distribution –virus of potato, a strains that causes formation on the tubers of circular necrosises ($PVY^{NTN}$) is educed [2]. The monitoring watching the phytopathogenic state of planting of potato are an important constituent in the complex of events that is sent to control and fight against vegetable pathogens. Such inspections give an opportunity to estimate the local zones of risk of distribution of viral diseases for agricultural cultures, to find out progress and changes of phytopathogenic situation trends in agrocoenosiss.

During realization of the phytopatogenic monitoring we followed methodology of inspection of sowing of potato [3], for the analysis of standards applied the complex of diagnostic methods: symptomatology, immunology, biotesting, electronic microscopy. In researches used detectors of immunity, made on the basis of strains of viruses of potato, natural isolates of viruses got at research.

Analysis of situation, that was folded in the seed-grower of potato shows that sorts are largely staggered by viruses. On results researches 2011 - 2015 in the nurseries of elite seed-grower of potato educed: M-, S -, Y-

viruses of potato, both in a monoinfection and in composition of pathogenic complexes.The entomophillous M-virus of potato predominates in sowing in a monoinfection (36 %) or in a complex with other tesselated viruses: PVM+PVS is educed in plants 24 %, PVM+PVS+PVY - 28 %, PVM+PVY - 6 %, PVS+PVY - 2 %, PVS - 4 % the inspected sorts.

Monitoring inspections showed a high level to reinfection of the revitalized seminal material of sorts Slovianka, Serpanok and Nevska. Distribution of viral diseases in clonal material presented 100 % on results serum analyses. In the clonal planting the PVM prevails in a monoinfection (48,1 %) or in a complex from PVS by the virus of potato (35,6 %), PVY is not educed.

On planting of potato in the agrocoenosiss of the Chernihiv area: PVY - is widely widespread, that is educed in plants 18 from analysed 47 sorts of potato (38 %). In the field terms of PVY is educed in the plants of potato with the symptoms of the spotted mosaic of different intensity wrinkled, and also, in plants without the external signs of disease. On the potato of different sorts looked after distribution of disease, that showed up development on the plants of the tesselated colouring of sheets of different intensity. Sick plants here did not show lag in a height. Intensive development of symptoms of mosaic showed up in the phase budding-flowering of plants, at an increase temperature. In plants with the symptoms of the spotted mosaic it is educed pathogenic complexes of PVY with PVM and PVS.

Thus, in the agrocoenosiss of the Chernihiv area there is an intensive infection of plants of potato by viruses, that allows to talk about a hard natural infectious background (a presence is in the environment of effective reservators and vectors of potato viruses). At growing of healthy feedstock in the field terms during 2-3 there is a high degree of staggered by his viruses of tesselated group. Determinations of risks of distribution of phytopathogenic viruses of potato on the basis of study of features of ecology of pathogen are necessary for providing of effective control of virus on all stages of growing of sorts (in the process of making healthy, at production of potato seeds, certification , determination of sorts quality).

1. D. Shpaar, U. Brut, T. Betzel et al. Zaschita rasteniy v ustoychivyh sistemahzemledeliya [Plant protection in sustainable land use systems]. — Torzhok : Variant, 2003. — vol. 2. — 374 p.

2. Shevchenko O.P. Rozpovsyudzhennya ta diagnostyka nekrotychnyh shtamiv Y-virusu kartopli na Polissi Ukrainy. [Distribution and diagnosis of necrotic strain of potato virus Y-Polissya Ukraine]. — Visnik HNAY. Roslinnictvo, selekcia i nasinnictvo, plodoovocivnictvo i zbereganna— Kharkiv, 2006. — № 5. — pp. 110–115.

3. Metodychni rekomendacii shhodo provedennya doslidzhen z kartopleyu [Methodical recommendations for conducting research on potatoes]. Institute of Potato UAAS. Nemishaeve, 2002. — 182 p.

# PHYLOGENETIC ANALYSIS OF CAPSID PROTEIN GENE PART OF *SOYBEAN MOSAIC VIRUS* DETECTED IN LEFT-BANK FOREST-STEPPE OF UKRAINE

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*Soybean mosaic virus* (SMV) is the most prevalent virus and is recognized as the most serious, long-standing problem in many soybean producing areas in the world. Infection by SMV usually results in severe yield losses, seed quality seedling viability reduction of soybean. It is both seed- and aphid-transmitted, and numerous strains have been identified that vary in symptom induction on soybean cultivars, vector transmission and antigenic properties. According to their disease reactions on differential soybean cultivars, collected isolates of SMV have been classified into various strains. In the United States, using two sensitive and six resistant soybean cultivars, Cho and Goodman (1979) successfully classified 98 SMV isolates into seven strains, namely G1–G7. The same differential system was also utilized in Korea, resulting in additional SMV strains such as G5H, G6H, and G7H identified [1]. In Japan and China, however, different sets of soybean cultivars were used as differentials, and isolates of SMV collected in these two countries were finally classified into five (A to E) and 21 (SC1 to SC21) strains, respectively.

Study of soybean viruses in Ukraine was conducted exclusively on the Right Bank of its parts [2]. However, detection of these viruses in a left-bank forest-steppe has not yet been carried out. To investigate the belonging to strain clusters and genetic relatedness of studied SMV isolate with already published sequences of this virus, we have conducted the RT-PCR. RT-PCR showed the presence of *Soybean mosaic virus* in the sample №17 from Poltava region (Pol-17), as evidenced by the presence in agarose gel amplicons of appropriate size (194 bp).

Phylogenetic analysis of the nucleotide sequence of the part of capsid protein gene (positions 9505-9649 bp) of SMV isolate from Poltava region (Pol-17) and 36 isolates and strains of this virus showed a 100% level of homology between the Ukrainian representative isolate with a number of Asian, European and American isolates. As is evident from the phylogenetic tree, isolate Pol-17 belong to the one cluster and has a common origin with Chinese isolates HB-S19, HB-S23, HB-S27, SX-Z, XFQ014, ChS, Iranian and M isolate of Polish and American strain 452 (Fig.). Topology of the tree constructed using nucleotide sequences of the part of SMV capsid protein gene was very similar to that recently described for compete genome sequences of SMV isolates [3]. According to this classification, 83 isolates were divided into four clades – from I to IV. According to it, isolate Pol-17, that has 100% homology with the Chinese isolate XFQ014, belongs to clade IV, together with Iranian isolates Ar33, Go11, Lo3 and Canadian isolates L, L-RB, NP-C-L, NP-L.

This is the first phylogenetic analysis of SMV isolate from the left bank of Ukraine. Earlier, Sherepitko et al. isolated SMV from soybean on the right bank of the country (Vinnytsia region). It was investigated that Ukrainian isolate SMV (UA1Gr) for sequences of the coat protein coding region and P1 coding region has strong genetic relationships with SMV-VA2 isolate which together were sorted in one clade with G2strain [2]. However, we cannot include Ukrainian isolate UA1Gr to our analysis because studied areas of CP gene do not overlap.

Results of our phylogenetic analysis as the data of many other authors [1, 2, 4, 5] testifies about absence of clear correlation between genetic differentiation and geographical origin that can be explained with the processes described for the SMV recombination between its various strains. So, Seo et al. [1] analyzed the 44 SMV isolates and strains and did not provide clear clues for classifying the SMV population according to geographical origins. All known isolates/strains they are largely divided into two geographic subpopulations, Far East Asia and North America.

Soybean originated in East Asia and has been cultivated for several thousands of years, and it was recently suggested that SMV, like soybean itself, originated in South East Asia when it diverged from WMV around1500 years ago, and further diverged into the soybean and *Pinellia* lineages around a 1000 years ago. However, in North America, soybean was first introduced in 1765 and has been widely spread there in the early twentieth century. As soybean is the natural host of SMV in fields, the history of the virus in North America is believed to be similar to that of soybean [1]. Comparison of nucleotide diversities of within- or between-population also revealed that little genetic differentiation has occurred between two geographical subpopulations. Thus, it is likely that few sequence diversity have yet accumulated in SMV population of North America.

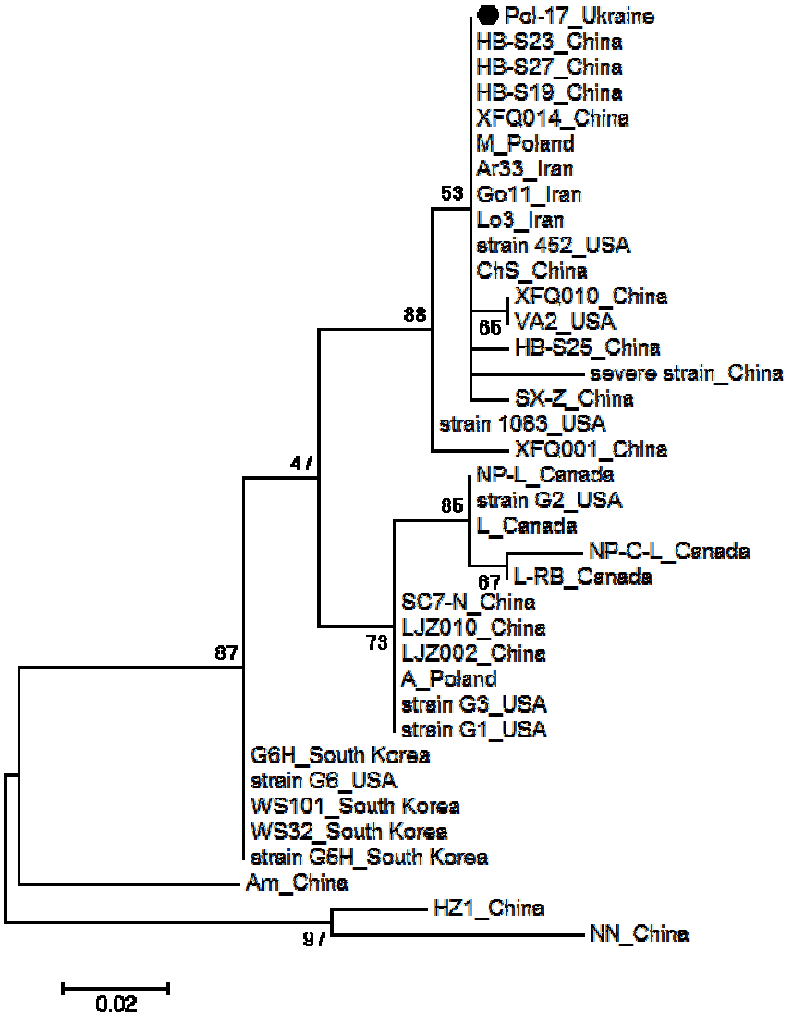


Fig. Maximum likelihood (ML) tree resulting nucleotide sequences of part of the CP gene of Ukrainian isolate Pol-17 and different isolates from other countries. The phylogenetic tree was constructed applying Jukes-Cantor model

This, taken together with our phylogenetic results, also suggests that not much genetic drift has occurred in the SMV population. On the other hand, the involvement of such human activities as trade of SMV-infected soybean seed or relatively recent spread of the virus from Asia on a

global scale can be another possible reason for phylogenetic inconsistency on geographical clustering [1]. The outcomes of these studies will certainly provide new insights into the evolutionary process of SMV in relation to its natural hosts.


1. Seo J.K., Lee H.G., Choi H.S., Lee S.H., Kim K.H. Infectious in vivo transcripts from a full-length clone of *Soybean mosaic virus* strain G5H. The Plant Pathology Journal. 2009; 25: 54-61.
2. Sherepitko D.V., Budzanivska I.G., Polischuk V.P., Boyko A.L. Sequencing and phylogenetic analysis of Soybean mosaic virus isolated in Ukraine. Biopolym. and Cell. 2011; 27(6):472–479.
3. Zhou G.-C., Shao Z.-Q., Ma F.-F., Wu P., Wu X-Y., Xie Z.-Y., et al. The evolution of soybean mosaic virus: An updated analysis by obtaining 18 new genomic sequences of Chinese strains/isolates. Virus Research. 2015; 208: 189-198.
4. Domier L. L., Latorre I. J., Steinlage T. A., McCoppin N., Hartman G. L. Variability and transmission of *Aphis glycines* of North American and Asian soybean mosaic virus isolates. Archives of Virology. 2003; 148:1925-1941.
5. Jezewska M., Trzmiel K., Zarzynrska-Nowak A., Lewandowska M. Identification of *Soybean mosaic virus* in Poland. Journal of Plant Pathology. 2015; 97 (2): 357-362.


## INFLUENCE OF PESTICIDES ON THE ACCUMULATION OF VIRUS IN THE SYSTEM "TOBACCO MOSAIC VIRUS – TOBACCO PLANTS"


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Development of plant viral diseases can cause significant yield losses [1]. Effective strategies to control these diseases may involve

eradication of the viral vectors (insects, nematodes, etc.) and wild plants (weeds), which can serve as reservoirs for the phytoviruses [2]. For the sake of struggle against the pests of plants and against the weeds, - aiming maximal increasing of the productivity, - agricultural chemistry generously uses different pesticides in our days: the fungicides, the herbicides, the insecticides, the defoliants, the desiccants, the regulators of the plants' growth, etc. Biological properties of the viruses, such as resistance and infectivity, depend on the conditions of their existence that is natural focus of viral infection. Type and development of the viral infection in plants depends on various factors. A plant, which is highly sensitive to virus under certain conditions, can become totally or partially resistant under the conditions of the transformed environment [3]. Nevertheless influence of the pesticides on the development of the phytoviral diseases is poorly researched. The problem of disease development actual research is because of spreading of the phytoviruses and intensive usage of the different types of pesticides at the agrarian and landscape sectors.

Common for phytoviral researches model system: tobacco mosaic virus (TMV) – plants of *Nicotiana tabacum* cv. Samsun» has been used in present study [4]. The preparations of Fundazol (fungicide), Fuzilad Forte (herbicide), Avercom, Avercom-nova (nematocides/ phytostimulators) and Actara (insecticide) were chosen accordingly with several criteria: first of all, they are commonly used in agriculture and at the urban landscape design in the cities of Ukraine, secondly, they are the pesticides of different origin and usage. The plants were sprayed with preparations twice in 7 days interval (before inoculation of by the virus). The plants of *Nicotiana tabacum* cv. Samsun were growing at the greenhouses with sterile ground. They were grown up under the standard conditions of the light and humidity regime and photoperiod (relative humidity - 40-50%, temperature - 24-28$^{o}$ C, photoperiod – 16 hours). The plants on the stage of four leaves were inoculated with the virus mechanically. An inoculation material was prepared using of 0,1M PBS, pH 7,4. The viral concentration was 200 mkg/ml. The enzyme-linked immunosorbent assay (ELISA) in the indirect modification was used to determine viral content in plants. The samples for ELISA were taken from each storey of the plants. The results were registered by the automatic ELISA-reader (Dynex Technologies, Germany) under the wavelength of 405 nm [5].

In summary, the pesticides Fundazol, Fuzilad Forte, Avercom, Avercom-nova, Actara displayed different effect on the development of systemic infection caused by TMV. At the early stages of the disease (the 10-th day after inoculation), the concentration of TMV is higher in the tobacco plants, which were sprayed by Fundazol, Fuzalid Forte, Avercom and Avercom-nova, than in the infected plants, which were not sprayed.

Applying the preparation of Actara causes an inhibition of the virus in the plants at the 10-th (in a 1,4 times) and 21-th day after inoculation (in a 1,2 times) in comparison with TMV infected control plants, but the TMV concentration increases during 10 days of disease in a 2,6 times. At the middle stage of TMV infection development (21-th day after infection the influence of all substances is the same: accumulation of virus is somewhat delayed, but none of the substances stimulate the increase of the virus content in plants.

So we can conclude that usage of the different types of pesticides doesn't prevent TMV accumulation during the middle stage of viral disease development. The fact, that the plants, which were grown up under influence of pesticides, can effectively support a systematic viral infection, even in the case of proper usage of the plants' protective means, expose certain danger as a possibility of a wide spreading of the viruses on the territory of Ukraine. Such researches were conducted for the first time. Their actuality is corroborated by published data on contamination of Ukrainian soils with pesticides [6]. It is reasonable to continue the investigations with widening the pesticides' spectrum and their concentrations as well as the spectrum of the phytoviruses.


1. Virusni ta mikoplazmovi khvoroby pol'ovykh kul'tur / Pid red. Zh.P. Shevchenko. — K.: Urozhay, 1995. – 304s.
2. Ambang Z., Ndongo B., Amayana D., Djilй B., Ngoh J. P., Chewachong G.M. Combined effect of host plant resistance and insecticide application on the development of cowpea viral diseases // Australian Journal of Crop Science. – 2009. – Vol. 3. – P.167-172.
3. Iutyns'ka O.A. Tsytomorfolohichni ta biolohichni osoblyvosti perebihu infektsiyi virusu tyutyunovoyi mozayiky za khronichnoho vplyvu ioniv vazhkykh metaliv.:dys. Kandydata biolohichnykh nauk / Iutyns'ka Olena Anatoliyivna. – Kyyiv. – 2010. – 20 s.
4. Mjet'juz R. Virusy rastenij: Per. s angl. – M.: Mir, 1973. – 600 s.
5. Hill S.A. Methods in plant virology. – Oxford: Alden Press, 1984. – 167p.
6. Baranovs'kyy V.A. Ukrayina. Ekoloho-heohrafichnyy atlas / V.A. Baranovs'kyy // Atlas-monohrafiya. – K.: Varta, 2006. – 220 s.

# CARLAVIRUS IN LILY COLLECTION OF GRISHKO' NATIONAL BOTANICAL GARDEN

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The genus *Lilium* is one of the most valuable commercial market flower bulbs in the world, mainly owing to its ornamental function as a cut flower or as a potted plant. Susceptibility of lilies to infectious diseases limits their popularity as ornamental plants. Fungi and bacteria are removed during the establishment of *in vitro* cultures from standard sterilization of bulb scales, whereas viruses are not [1].

Virus diseases of lily are of great significance because even when present in the latent state, the viruses could be transmitted to healthy plants and cause commercial losses [2]. In spite of the fact that we could predominantly diagnose virus infection basing on specific symptoms such as ring spot, mosaic and necrotic lesions, the identification of the pathogen is not possible. Sometimes lilies do not manifest signs of virus infection [3]. Besides, some factors such as disbalance of mineral nutrition, non-compliance with the light regime, invasion by insects and mites, infections caused by bacteria, mycoplasms and fungus, or genetic disorders could lead to symptoms similar to those of virus nature. This involves necessity for serological diagnostics of the collections for preservation of their commercial value.

Screening of *Lilium* plants for viral diseases in the collections of M.M. Grishko' National Botanical Garden was conducted. Different virus-like symptoms were detected on lily plants: mottling, mosaic, color breaking and leaf deformation. Infectious nature of disorders was confirmed using indicator plants typical for viruses normally infecting lilies. To determine virus nature of disease we conducted indirect and DAS-ELISA tests [4]. Same samples were analyzed in electron microscopy at 30,000 magnification.

Results of ELISA tests showed positive reactions of *Lilium* cultivar 'Royal gold', 'H-dawn' and 'Krema-2' with antiserums to *Potato virus S* (PVS). We deem it could indicate contamination of these plants with *Lily*

*symptomless virus* (LSV), which is serologically related to PVS [5].

Filamentous virus particles about 650 x 20 nm in size were observed in the sap of the plants. In addition, icosahedral particles were also observed, with a mean diameter of approximately 30 nm. Basing on serological, biological and morphological properties, we suggest that filamentous virus is related to *Lily symptomless virus*. Another virus is not completely identified, but symptoms induced on indicator plants suggested that it was *Tomato aspermy virus*.

Virus infections, especially latent diseases, are very dangerous because of the extensive exchange of untested plants among different botanical gardens and private collections. Additionally the incidence of such viruses as LSV which are generally symptomless in field-grown plants may cause problems while plants infected by other viruses. Besides, vegetative propagation of lilies without virus monitoring leads to uncontrolled distribution of viral infections within the collection.

1. Nesi B., Trinchello D., Lazzereschi S., Grassotti A., Ruffoni B. Production of Lily symptomless virus-free plants by shoot meristem tip culture and in vitro thermotherapy // Hortscience. -2009. - 44(1). –P.217–219.
2. Farzadfar Sh., Pourrahim R., Torkian M., Maleki M. An investigation on characterization of cucumber mosaic virus isolated from lily green house in Damavand County, Iran // Iranian Journal of Virology. -2014. -8(2,3). –P: 36-43.
3. Cees J.A. Control of aphid-borne Lily symptomless virus and Lily mottle virus in Lilium in the Netherlands // Virus Research. -2000.- 71 (1,2). –P. 23–32.
4. Clark M.F., Adams A.N. Characteristics of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant viruses // J Gen Virol.- 1977.- 34. –P.474-83.
5. Genus Carlavirus. In: M.Q. King A., Lefkowitz E., Adams M. J., Carstens E. B. Virus Taxonomy. Ninth report of the International committee on taxonomy of viruses. Academic Press, San Diego, 924-927.

# MOLECULAR COMLEXIS CONTAINIG GLYCANS, RHAMNOLIPIDS AND THIOSULFONATES AS ANTIVIRAL AGENTS

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Our previous studies have shown that some glycans (Gs) obtaining from higher medical mushroom *Basidiomycota* and yeasts have a broad spectrum of biological activities, including antiviral ones (Kovalenko et al., 2009, 2010a, 2010b, 2013). Moreover, as experiences show, Gs activity increases in the presence of biosurfactants (BS). Meanwhile, there are some problems of the Gs as polymers delivery to their targets, particularly in plant cells equipped with rigid cuticle and cellulose cell wall.

Therefore, studies aimed at finding of substances which promote the penetration of active biopolymers to plant tissues are topical modern problem. One of approaches to solving this problem is the applying of liposomes (LS). The experience in the creation of LS-based microbial BS of rhamnolipid nature (RL), produced by bacteria of the genus *Pseudomonas* is already available.

Another interesting object for creation of LS are thiosulfonate (TS) – synthetic analogues of natural allicines, which have antimicrobial and antiviral activity (Lubenets et al, 2013; Navrot et al, 2012; Sotirova et al, 2012). Since TS is lipophilic substances, in certain conditions they can form liposomes with amphiiphilic molecules RL.

We used the method of creating LS from rhamnolipids (Pat. US 4,902,512). We have optimized the conditions of LS formation: the temperature of phase transition RL, the parameters of ultrasonic processing, the degree of inclusion of glycopolymers in liposomes, the ratio of Gs, RL TS; LS compositions, molarity and pH values of buffers; stability of obtained multimolecular complexes.

The stability of obtained LS has installed in different manipulations (autoclaving, centrifugation, lyophilization), as well as during storing at various temperatures. The biological activity of LS was studied using seeds of radish, tobacco, tomato, wheat and plants of tobacco, datura, soy, beans, potato tubers. As models of viruses were used tobacco mosaic virus, viruses of soy, potato, wheat, alfalfa and other agricultural crops.

The results of experimental studies showed increasing of antiviral activity of natural Gs in compositions of the created liposomes. These results can be explained by the fact of promotion of the active substances penetration in plant tissues. The designed LSs contribute increasing of productivity and improving of the structure of field crops productivity. LS as was shown in our work has not any phytotoxicity in concentrations, that are effective against studied viruses.

Thus, the results of investigation of liposomes containing on Gs, RL and TS indicate perspectives of their using for further improving of the targeted delivery biological active substances.

# DISCRIMINATION OF BEAN YELLOW MOSAIC VIRUS UKRAINIAN ISOLATES WITHIN THE COAT PROTEIN–DERIVED PHYLOGENETIC GROUPS

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Bean yellow mosaic virus (BYMV) occurs worldwide and infects a wide range of legume species as well as some non-legumes [1]. There are many strains of BYMV infecting various crops in different parts of the world. The seven distinct strain groups were proposed [2] to subdivide the BYMV strains in relation to original natural isolation hosts and geographical distribution. There were “monocot”, “lupin”, “canna”, “broad bean” and “pea” groups. The monotypic group called “W” consisted of Isolate W from *Lupinus albus* and the largest group named the “general”

contained isolates obtained from dicotyledonous (*Fabaceae*) and monocotyledonous (*Iridaceae*, *Orchidaceae*) families. In Ukraine two isolates of BYMV originating from soybean (Glycine soya) and bean (*Phaseolus vulgaris*) plants were obtained and examined according to their molecular characteristics. Sequences of Ukrainian isolates from soybean and bean hosts were compared with those of 34 CP sequences available on the database the molecular variability according their phylogenetic groupings.

Genetic diversity of isolates was deduced from nucleotide sequence alignments in MEGA 6 [3] using neighbor-joining method with arithmetic mean. Nucleotide sequences of BYMV isolates used in comparison were retrieved from Genbank entries: pea (AB032023, AB041971, D00604, S71232, DQ641248), Monocot (AB079782, AB097089, AF185961, AJ311371, AY845011, AY845012, EU082118), General (AB097090, AF192783, DQ901435, EU082121, EU082122), Canna (EF592168, EF592169), Broad bean (EU082113, EU082114, EU082119, EU082120, EU082128, KF823012), Lupines (EU082117, EU082124, EU082125, EU082126, EU082127, EU144223) [2]. The sequence data of the Ukrainian BYMV isolates have been submitted to NCBI previously with accession numbers KT923790.1 for soybean isolate and KT923791.1 for bean one. Clover yellow vein virus (ClYVV) isolate (AB011819) was used as the outgroup sequence. It should be noted that only two sequences of BYMV isolates derived from soybean hosts were available in database (KC731531, Australia; KJ872537, India).

The phylogram showed (Fig. 1.) that the soybean isolates were very close to the lupine group as well as to general one – the largest group containing isolates from different species of dicotyledonous and monocotyledonous families and originally collected in Asia, Australia, Europe, and North America from wild or domesticated species of legumes, wild orchid species, and the domesticated species Gladiolus hybrida [2]. Phylogenetic analysis of the CP sequences revealed a separate distinct clade consisting from soybean isolate sequences. Interestingly, Ukrainian BYMV isolates from the soybean and bean belong to the same phenotypical "soybean" group even though they strongly differed in their pathogenicity on the test plant and were referred as necrotic and non-necrotic types of strain respectively [4]. It can be explained by the fact that CPs of potyviruses are involved in the host-encoded susceptibility [5] and the genome-linked viral protein (VPg) has plays role in host range determination [6] suggesting that their CPs evolved differently from their VPgs. The CP-determined grouping of isolates obtained are of considerable interest in the understanding of BYMV strain evolution taking into account the very strong geographic remoteness isolates analyzed in this work. It is possible BYMV strain group specialization of viral strains occurred

independently on each continent and defining role in this process belongs to the recombination.

1. King A.M.Q., Lefkowitz E., Adams M.J., Eric B.C. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses Elsevier /Academic Press, San Diego, 2011

2. Wylie S. J., Coutts B. A., Jones M., Jones R. Phylogenetic analysis of Bean yellow mosaic virus isolates from four continents: Relationship between the seven distinct groups found and their natural isolation hosts and geographical origins. Plant Dis. - 2008. – 92. – p. 1596-1603.

3. Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods // Mol. Biol. Evol. – 2011. – 28, 10. – p. 2731-2739.

4. Kyrychenko A., Antipov I., Grynchuk K. Ukrainian BYMV isolates: various pathogenicity, the same nucleotide sequences of coat protein region.: Abstracts book II International Scientific Conference «Microbiology and Immunology – the development outlook in the 21st century». 2016 April 14-15; Kyiv: 2016. p. 87.

5. Hofius D., Maier A. T., Dietrich C. et al. Capsid protein-mediated recruitment of host DnaJ-like proteins is required for Potato virus Y infection in tobacco plants. J. Virol.- 2007.- 81. – p. 11870-11880.

6. Rajamдki M.L., Valkonen J.P.T. The 6K2 protein and the VPg of Potato virus A are determinants of systemic infection in Nicandra physaloides. Mol. Plant-Microb. Interact. -1999. -12. – p. 1074-1081.

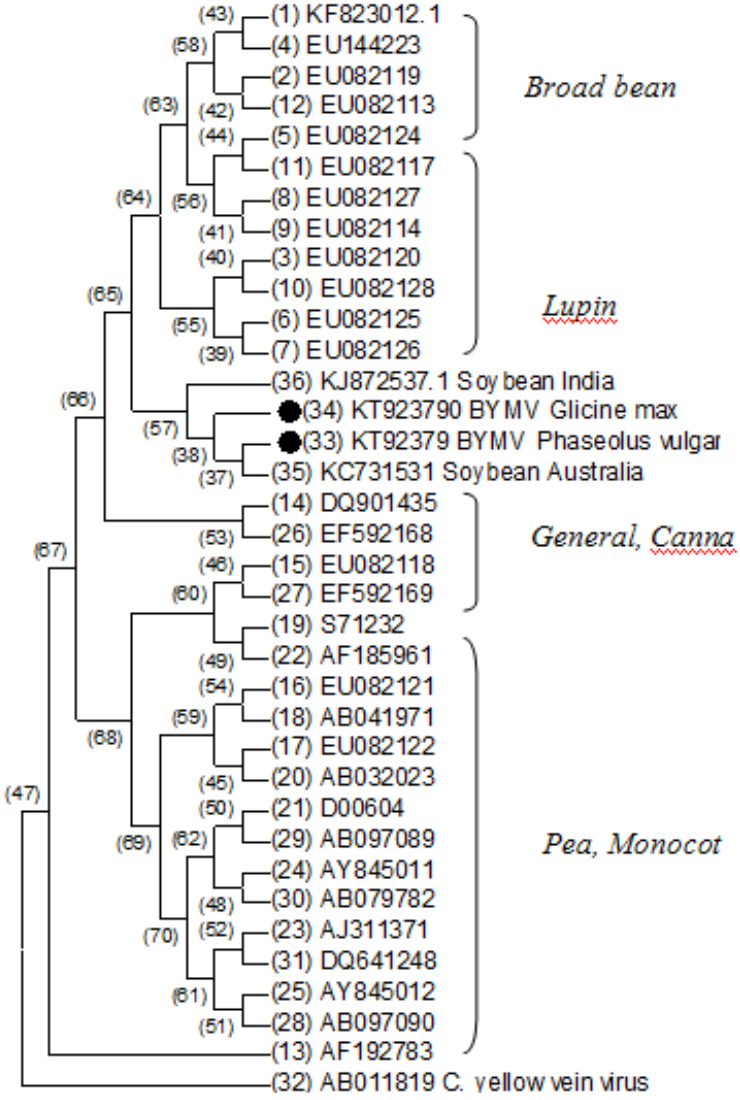


Fig. 1. Neighbor-joining relationship phylogram of the coat protein nucleotide sequences of 36 BYMV isolates. Names of the polytypic groups are based on natural hosts. Numbers at branches indicate the percentage of 1,000 bootstrap replications

# SIMILAR CONSERVATIVE MOTIFS IN TOBAMOVIRAL SUBGENOMIC AND GENOMIC PROMOTERS

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*Tobamovirus* is the largest genus in the family *Virgaviridae*, which consists of 29 definitive and 6 unclassified species [1].The type member of the tobamoviruses, tobacco mosaic virus (TMV), contains a monopartite single-stranded positive sense genomic RNA that encodes four proteins [2]. The replication 126K and 183K readthrough proteins are translated from genomic RNA, and both the movement protein (MP) and the coat protein (CP) – from their subgenomic mRNAs, which are initiated internally at the subgenomic promotors (SGPs) on a negative-strand template [3, 4]. Untranslated regions (UTRs) located at the 5′ and 3′ termini of the genomic RNA contain genomic promotors (GPs) required for initiation of minus- and plus-sense RNA synthesis [5, 6]. Replication takes place in cytoplasmic viral factories [7].

In contrast to the detailed knowledge of negative-strand and subgenomic RNAs promotion, genomic positive-strand RNA initiation remains poorly defined. Because of this, we have undertaken the computational searching of similar conservative sequences in genomic RNAs of 17 TMV strains and 21 tobamovirus species.

In genomic sequences of plus-strand RNAs we have revealed 9-nt motifs GGTTCGTTT and GATTCGTTT, located upstream of the translation start codon of coat protein (Cp) and movement protein (Mp) genes, respectively. These motifs correspond to CCAAGCAAA and CTAAGCAAA in minus-strand RNAs, which were found in tobamoviral subgenomic promoters [4], and are similar to the ICR2 consensus of tRNA gene promoters, GGTTCGANTCC, that are required for promotion of minus-strand synthesis of viral and eukaryotic RNAs [8, 9]. Stem-loop S3L3 of domain D1 in the 3' untranslated region of TMV-L stain, GGGATTCGAATTCCC, that are required for both promotion of minus-strand RNA synthesis and binding to the RNA polymerase in vitro [10] also contains GATTCGTTT-like motif. In contrast to this, no similar conservative motifs we have found in 38 tobamoviral promoters of plus-strand RNA synthesis.

In the light of these data we hypothesize that semipalindromic GGTTCGAATCC-like motifs may be involved in the initiation of both plus and minus genomic RNA synthesis via the common TTCGAA site (Table 1). Two complementary nucleotides flanging TTCGAA appear to be required for a stem-loop secondary structure, which is found to be essential for promoter activity (4, 11, 12).

**Table 1.** Similar ICR2-like motifs on plus and minus strands of tobamoviral genomic RNAs

| Motifs [1] | RNA strand | Correspondence with GGTTCGAATCC [2] | Genomic positions [3] | Number | |
|---|---|---|---|---|---|
| | | | | TMV strains | Tobamoviruses |
| GG**TTCGAA**TCC | + | 11 | -36, +26 | 11 | 9 |
| GGA**TTCGAA**CC | - | | -35, +25 | 1 | 6 |
| | | | -22, +12 | 1 | 0 |
| Ga**TTCGAA**TtC | + | 9 | -36, +26 | 4 | 2 |
| GaA**TTCGAA**tC | - | | -35, +25 | 0 | 1 |
| tG**TTCGAA**TCa | + | 9 | -37, +27 | 0 | 1 |
| tGA**TTCGAA**Ca | - | | -38, +28 | 0 | 2 |

[1] White background indicates positive sense sequences, gray background – negative sense, bold font – common site in all tobamoviruses tested, small letters – complementary nucleotides substitutions. [2] Motifs' nucleotide numbers coinside with `GGTTCGAATCC.` [3] Motifs' positions relative to the 3' end of plus-strand RNA (-22...-38) or to the 5' end of minus-strand (+12...+28)

Our results suggest that both Cp and Mp subgenomic promoters as well as genomic promoters for minus- and plus-strand RNA synthesis contain similar nucleotide motifs, that are conservative among tobamoviruses. The presented data are in agreement with those of Grdzelishvili et al. [4] who map the boundaries of the TMV Cp and Mp sgRNa promoters, reexamined the transcription start site for the Mp sgRNA and reveal same sequences and putative secondary structures important for sgRNA promoter activity. However, our data do not support a key role of secondary structures, but not its sequences in SGPs activity [4] so far as MP SGP contains two stemloops but CP SPG only one and quite different stemloop, as well as both SGPs and both GPs maintain similar GGTTCGTTT-like motifs.

These contradictions can be explained by the complex regulatory system involving in promoter recognition and RNA synthesis. It includes a series of sequence and secondary structures, different long-range RNA-RNA and RNA-protein interactions, numerous host factors as well as linear, extended duplex and circular RNA conformations [13, 14, 15].


1. Adams M.J., Antoniw J.F., Kreuze J. Virgaviridae: a new family of rod-shaped plant viruses. Arch. Virol. - 2009.-154. – p.1967-1972.
2. Goelet P., Lomonossoff G.P., Butler P.J.G., Akam M.E, Galt M. J., Karn J. Nucleotide sequence of tobacco mosaic virus RNA. Proc Natl Acad Sci, USA. – 1982. - 79. – p. 5818-5822.
3. Beachy R.N., Zaitlin M. Characterization and in vitro translation of the RNAs from less-than-full-length, virus-related, nucleoprotein rods present in tobacco mosaic virus preparations. Virology. – 1977. – 81. – p.160-169.
4. Grdzelishvili V.Z., Chapman S.N., Dawson W.O., Lewandowski D.J. Mapping of the Tobacco mosaic virus movement protein and coat protein subgenomic RNA promoters in vivo. Virology. – 2000. - 275 (1). – p. 177-192.
5. Chujo T., Ishibashi K., Miyashita S., Ishikawa M. Functions of the 5'- and 3'-untranslated regions of tobamovirus RNA. Virus Res. – 2015. - 206. – p. 82-89.
6. Dreher T.W. Role of tRNA-like structures in controlling plant virus replication. Virus Res. – 2009. - 139(2). – p. 217-229.
7. Harak C., Lohmann V. Ultrastructure of the replication sites of positive-strand RNA viruses. Virology. – 2015. - 479-480. – p. 418-433.
8. Marsh L.E., Pogue G.P., Hall T.C. Similarities among plant virus (+) and (-) RNA termini imply a common ancestry with promoters of eukaryotic tRNAs. Virology. -1989. – 172(2)– p.415-27.
9. Pogue G.P., Hall T.C. The requirement for a 5' stem-loop structure in brome mosaic virus replication supports a new model for viral positive-strand RNA initiation. J. Virol. - 1992. - 66. – p. 674-684.
10. Osman T., Hemenway C., Buck K. Role of the 3' tRNA-Like Structure in Tobacco Mosaic Virus Minus-Strand RNA Synthesis by the Viral RNA-Dependent RNA Polymerase In Vitro. Journal of Virology. - 2000, 74 (24). –p. 11671-11680.
11. Li W., Wong S.M. Analyses of subgenomic promoters of Hibiscus chlorotic ringspot virus and demonstration of 5' untranslated region and 3'-terminal sequences functioning as subgenomic promoters. J Virol. – 2006. – 80 (7). – p. 3395-405.

12. Skov J., Gaudin M., Podbevsek P., Olsthoorn R.C., Petersen M. The subgenomic promoter of brome mosaic virus folds into a stem-loop structure capped by a pseudo-triloop that is structurally similar to the triloop of the genomic promoter. RNA. - 2012. -18 (5). - p. 992-1000.
13. Gunawardene C.D., Jaluba K., White K.A. Conserved motifs in a tombusvirus polymerase modulates genome replication, subgenomic transcription, and amplification of defective interfering RNAs. J Virol. – 2015. - 89 (6). – p. 3236-3246.
14. Kawamura-Nagaya K., Ishibashi K., Huang Y.P., Miyashita S, Ishikawa M. Replication protein of tobacco mosaic virus cotranslationally binds the 5' untranslated region of genomic RNA to enable viral replication. Proc Natl Acad Sci US. – 2014. - 111(16). – p. 1620-1628.
15. Nagy P.D., Pogany J. Global genomics and proteomics approaches to identify host factors as targets to induce resistance against Tomato bushy stunt virus. Adv Virus Res 2010.-76.123-177.

## DETECTION OF THE PATHOGEN OF VIRAL DISEASE IN *SAMBUCUS NIGRA* PLANTS

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Elderberry (*Sambucus nigra* L.) is widely used in scientific and traditional medicine [1]. Extracts of elder berry flowers are part of complex

drugs as "Novo Passit", "Sinupret", "Atma", etc [2]. It is known that the therapeutic activity of elderberry fruit is second only to chokeberry, which superior elderberry fruit for antioxidant effect. Biologically active compounds of elderberry fruit, flowers, leaves have antiviral, antibacterial, anti-inflammatory, analgesic, immunomodulatory and antiproliferative effects. Traditional medicine recommends taking elderberry fruit with damage to the mucosa of the stomach, liver and pancreas. Tincture of the flowers and leaves of black elderberry has anti-inflammatory, antioxidant and hepatoprotective activity [3].

*Sambucus nigra* L. plants are sensitive to atmospheric drought. Elderberry propagated mostly by seeds. Elderberry flowers contain up to 82 mg% ascorbic acid, glycoside sambunigrin, rutin, essential oils, organic acids, anthocyanins, phenolic compounds, coumarin, triterpenoids, micro elements, etc [2]. It is known that the content of ascorbic acid and essential oil in the raw material depends on the illumination of elderberry growth place. Ascorbic acid content in the raw material collected at cutting down was higher by 20% and amounted to 75-82 mg% compared with raw materials collected from plants undergrowth. Essential oil content indicators were also higher - by 10-15%. Essential oil content within the same version of the raw material varies depending on the lighting stage, from which were collected materials. Essential oil content can vary from 0.03% to 0.14% depending on the placement of flowers on the plant. Wild elderberry plants are good spring and summer honey.

On the chemical composition and content of biologically active substances in medicinal plants significantly affect pests and diseases, including viral [4]. It is known that various elderberry species infected by viruses that affect the metabolism of plants, reduce productivity and can degrade the quality of medicinal raw materials. There is report about infecting of *Sambucus canadensis* plants by filamentous virus which similar on morphological features to carlavirususes [5]. Subsequently, the virus was detected in the Netherlands and was named *Elderberry virus A* [6]. Recent studies of elderberry samples (*Sambucus* spp.) from Missouri (USA) showed infecting of these plants with two different viruses, which also belong to the genus *Carlavirus* [7]. In addition, *Tomato ringspot nepovirus* was detected in elderberry plants with symptoms of viral infection [8]. It was also discovered strain of *Cherry leaf roll virus*, which infected *Sambucus nigra f. aurea* (Sweet) Schwer., and was differ on serological properties from other known strains of the virus [9]. In North America *Cherry rasp leaf virus* (CRLV) was detected in elderberry plants exhibiting chlorotic ring patterns, leaf blotch, and leaf deformations [10]. Five novel carlaviruses with genome contig length ranging from 6044 to 8749 nt (GenBank accessions KJ572560–KJ572564) were discovered. All are new

viruses given that the complete RdRp genes share less than the 80% amino acid sequence identities (ICTV species cut-off value) between them and other members of the genus. The viruses are provisionally named as elderberry carlaviruses A–E [11]. Elderberries are part of the native vegetation and could act as potential reservoirs of CRLV and play an essential component in the management of the virus in cherry production areas [10].

The observations of wild elderberry plants in Poltava (2015) and Kyiv (2016) regions were detected plants with chlorotic symptoms and rolling of leaf tops and twisting up the edges of the leaves.

Number of affected plants accounted for over 20% of surveyed wild elderberry. That's why the aim of our research was to study the agent of the revealed disease. Filamentous virions 600 Ч 12 nm were found in the elderberry leaves conducting the transmission electron microscopy method. It was marked higher concentration of virions in plants with leaf rolling symptom compared with chlorotic.

This is the first report about viral disease of elderberry plants in the Ukraine. Detected virus deserves on further virological investigations to identify the pathogen because it can be serious problem for other economically important crops.


1. Chekman I.S. Clinical phytotherapy. – К.: «Rada», 2006. – 628 p. *In Ukrainian*
2. Velma V.V. Phytochemical study of the genus Sambucus and development of drugs on their basis: PhD thesis. Kharkiv, 2008. – 20 p. *In Ukrainian*
3. Vlachojanis J.E., Cameron M., Chrubasik S. A systematic review on the sambuci fructus effect and efficacy profiles // Phytother Res. – 2010. – V. 24(1). – P. 1-8.
4. Mishchenko L.T., Dunich A.A., Dashchenko A.V., Polischuk V.P. Viral diseases of medicinal plants. – К.: Phytosociocenter, 2015. – 320 p. *In Ukrainian*
5. Jones A.T. Purification and properties of elderberry latent virus one of four obtained from American elder (*Sambucus Canadensis*) // Ann.Appl. Biol. – 1972. – V.70. – P. 49-58.
6. Van Lent J.W. M., Wit A.J., Dijkstra J. Characterization of a Carlavirus in Elderberry (Sambucus spp.) // Neth. J. PI. Path.– 1980. – V.86. – P. 117-134.
7. Keller, K.E., Mosier, N.J., Thomas, A.L., Quito-Avila, D.F. and Martin, R.R. (2015). Identification of two new Carlaviruses in Elderberry // Acta Hortic. – Vol. 1061. – P. 161-164.

8. Uyemoto J.K., Gilmer R.M.Sap transmissible viruses from elderberries in New York // Phytopatology. – 1971. – V. 61(2). – P. 132.
9. Jones A. T., Murant A.F. Serological relationship between cherry leaf roll, elm mosaic and golden elderberry viruses // Ann. appl. Biol. – 1971. – V. 69. – P. 11-15.
10. Villamor D., Eastwell K. *Sambucus nigra* subsp. *caerulea* and *Malva* spp.: Newly Identified Hosts of *Cherry rasp leaf virus.* // Plant Disease. – 2016, Vol.100(4). – P. 867.
11. Ho T., Tzanetakis I. Development of a virus detection and discovery pipeline using next generation sequencing // Virology. – 2014. –Vol. 471-473. –P. 54-60.

# MONITORING AND ECONOMIC JUSTIFICATION OF PREJUDICE INTO FORCE ON VIRAL DISEASES OF MEDICINAL PLANTS IN THE CONDITIONS OF FOREST ECOSYSTEMS POLISSIA

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The results of research spread of viral diseases of medicinal plants in the forest phytocenoses. The paper was applied modern virological, environmental, alelopatic studying methods of identification of pathogens and their ability to induce pathological processes in different species of medicinal plants. The objects of research were used: hops (*Humulus lupulus*); *Betula pendula* (warty) (*Betula pendula*, or B. Verrucosa); Forest strawberry (*Fragaria vesca*); Plantain Large (*Plantago major*); Plantain Pollen (*Plantago lanceolata*) [1].

As additional types of co-analyzed used pine, maple, aspen and some herbaceous plants. In experiments using viruses' plant indicators fluorescent, electronic microscopy, IFA [2]. These screening plants conducted in the dynamics of their growth and development.

The economic justification harmfulness pathogens were based on performance, solutions plantain leaf surface under conditions VTM isolates lesions, weight indicators of forest plants of strawberry (ilarvirus); inflorescence formation and hops infected of carlovirus, ilarvirus (often it is a latent infection) growth and development (plant height) Betula Pendula at the age of five affected VOM. It is noted, the formation of symptoms in forest ecosystems medicinal plants have certain synchronous and cyclical exposure. They are 15-28%. Some viruses appears in the analysis of the survey results have a circular correlation with agrocenoses surrounding crops. As a result of research work there are compiled schematic map in terms of distribution phyto-viruses of Woodlands, economic evaluation criteria developed economy medicinal plant forest plant viruses in lesions of different taxonomic groups. The technique of determination economics impact of healthy plants perform virus used that is in mushroom [3]. There has been a marked a positive return on productive receiving raw materials from medicinal plants, created computer database results that based on economic indicators.

1. Polishchuk V.P., Budzanivska I.G., Ryzhuk S.N., Patyka V.P., Boyko A.L., 2011. Monitoring of plant virus infections of biocenoses of Ukraine, Phytosociocentre, Kyiv, 220 pp.
2. Virus taxonomy. Ninth report of the International Committee on Taxonomy of Viruses / Eds. Andrew King, et al. – Wien: Springer-Verlag, 2012. – 1327 p
3. Boyko O.A. Study of edible and medicinal mushroom viral diseases // Int. J. of Medicinal Mushrooms. – 2001. – 3. – P. 123.

# PHYTOSANITARY MONITORING *PLUM POX VIRUS* IN ODESSA REGION

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Plant viruses are group of pathogens that cause important loses in different fruit crops and they have great economic importance. One the most important virus on many *Prunus* species, causing great economic losses is *Plum pox virus* (PPV), causal agent of Sharka disease. Since its discovery, Sharka has been considered as a calamity in stone orchards. [1]

On the territory of Ukraine there are 5 areas under the quarantine and the total area of plum pox virus infection is more than 4 thousand ha. Odessa region takes the third place in Ukraine in PPV disease.

This work was aimed at conducting Sharka disease monitoring of planted stone fruit crops in Odessa region using visual, serological and molecular methods for further use by plant quarantine services, virologists and producers. For the 5 years (2010-2014), plant disease monitoring of 760 hectares of stone fruit orchards in Odessa region was conducted.

Visual diagnostics of PPV-specific symptoms was followed by serological analysis in the laboratory. Collected samples were tested for PPV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Bioreba (Switzerland) following the manufacturer's recommendations. RT-PCR was mainly used as a confirmatory test for selected samples using probes specific to PPV-D and PPV-M strains. In 2010-2014, routine ELISA diagnostics of such collected stone fruit samples showed that the percentage of PPV-infected trees increased annually. In Odessa region, plum was the most infected culture, and peach was less affected

In 2010, disease symptoms were detected in 54,4% of all observed plum trees and in 19,6% of peach trees, while in 2014 the percentage of infected trees was reached 78,6% and 40,5%, respectively. By January 1,

2010, the area of PPV spread in the Odessa region has increased by 8 hectares.

Therefore, the results of 5-year PPV monitoring in the affected areas of Odessa region revealed high occurrence of PPV and, more importantly, a positive tendency of virus spread. Obviously, these outcomes proved the ineffiency of the applied quarantine measures to contain PPV spread in Odessa region in particular and in Ukraine in general.

Six hotbeds of PPV infection totalling 28 hectares were found in Odessa region.

In turn, these results underline the need for

1) regular screening of stone fruit orchards (commercial, suspect/endangered, and quarantined ones as minimum) for PPV using EPPO-approved serological and/or molecular techniques,

2) a database system combining virus monitoring, crop, vector, climate data.


1. Kostadinovska E, Mitrev S.(2016) Plum pox virus strains, presence and distribution on different fruit species in Macedonia. The Book of Abstracts III International Symposium on Plum pox virus, Antalya-Turkey 9-13 May, 2016 p. 43
2. Clark, M. F., & Adams, A. M. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. The Journal of General Virology, 34, 475–483. doi:10.1099/0022-1317-34-3-475.


## DYNAMICS OF SPREAD OF HVX IN HOSTA PLANTS COLLECTION OF GRYSHKO' NATIONAL BOTANICAL GARDEN


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*Hosta virus X* was first identified and described in 1996 in Minnesota, USA. Since then, HVX has been reported from other US states

(Connecticut, Illinois, Indiana, Iowa, Kansas, Maryland, Massachusetts, Michigan, Ohio, Pennsylvania, Tennessee, Virginia, Wisconsin), Canada (British Columbia, Quйbec, Ontario), from EPPO region **(**Czech Republic, Finland, France, Italy (transient, found once and all infected plants were destroyed), Netherlands, Poland), Asia **(**China (Beijing), Republic of Korea), and Oceania (New Zealand (North Island)) [1]. In Ukraine, HVX was first detected in Kyiv back in 2012 [2].

The virus is easily transferable from one plant to another by mechanical means and as a result of vegetative propagation. In hostas, HVX symptoms include leaf mosaic, mottling, interveinal chlorosis between secondary veins, deformation and desiccation. Often infected plants exhibit reduced growth and dieback. Colour-breaking can also be observed on flowers of infected plants. HVX can also remain latent in infected plants for years without showing symptoms [3-6]. Among the viruses infecting hosta, HVX has the most economic impact [7].

In this report, we studied the dynamics of spread of HVX in Hosta plants collection of Gryshko' National Botanical Garden.

Gryshko' National Botanical Garden's *Hosta* plants collection is the most comprehensive in Ukraine, annually filling up with new plants from the Netherlands and Poland.

The plants isolates used in this study were obtained from different symptomatic cultivars before the vegetation season. For testing, we selected hosta cultivars with different morphological characteristics including leaf color, pattern, size and year of vegetation.

The collection was screened in 2013 and 2016 by ELISA and PCR. In 2013, 45% of selected samples were infected by HVX. In 2016, results of screening showed 25% of HVX-infected samples among 125 samples. Interestingly, 10% of infected plants were imported and introduced into the collection in 2016. The total number of screened samples reached 173.

The results show that HVX spreads unstoppably through plant collection from year to year. HVX-resistant plant species were also infected with HVX, probably due to the breaking of virus resistance over the time which could be a reason of high speed of pathogen spread.

Our study demonstrates the necessity for checking *Hosta* plants for HVX to prevent its epidemic spread.

1. EPPO RS 2013/175 https://www.eppo.int/QUARANTINE/Alert_List/viruses/HVX.htm
2. G.Shchetynina, I.Budzanivska, A. Kharina, Pereboychuk. First detection of Hosta Virus X in Ukraine // Вісник Київського національного університету імені Тараса Шевченка. 201. 2012 – Vol. 62 – P. 48-50.

3. Currier, S., and Lockhart, B. E. L. 1996. Characterization of a potexvirus infecting Hosta spp. Plant Dis. 80:1040-1043
4. Lewandowski, D. J. 2008. Hosta virus X. Online publication/HYG-306908. http://online.osu.edu/hyg-fact/3000/pdf/3069.pd
5. Ryu, K. H., Park, M. H., and Lee, J. S. 2002. Occurrence of mosaic disease of hosta plants caused by Hosta virus X. Plant Pathol. J. 18:313-316
6. EPPO RS 2013/175 https://www.eppo.int/QUARANTINE/Alert_List/viruses/HVX.htm
7. Carola M. De La Torre. 2012. Biological and Molecular Characterization of a U.S. Isolate of Hosta virus X. PHYTOPATHOLOGY J 1176 – 1180.

# PRODUCTION OF DIAGNOSTIC ANTISERA SPECIFIC TO BEET NECROTIC YELLOW VEIN VIRUS

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Sugar beets are unique high productive and profitable crop. In Ukraine sugar beet is the most important technical crop which is the only source of raw material for sugar industry. Rhizomania is one of the most serious diseases of sugarbeet. Rhizomania can greatly reduce sugar yield by reducing either the tonnage or sugar content, or both tonnage and sugar content, of harvested roots. The causal agent of rhizomania is Beet necrotic yellow vein virus (BNYVV) and a common soilborne fungus serve as the vector or carrier for this virus. Although the virus is quarantine, it is distributed in different regions of Ukraine. Control of this pathogen is complicated due to long-term survival of virus (more than 10 years) in fungal spores. As fungal spores protect virus from inactivation, the chemical control arise as inappropriate means from an environmental and economic

points of view. The only effective way to combat this virus is to use resistant plant varieties and hybrids. The aim of our work was the production of diagnostic antisera specific to BNYVV for further identification of virus on different stages of plant breeding and surveys of virus distribution among industrial crops of sugar beet in Ukraine applying the enzyme-linked immunosorbent assay.

Virus-infected beet plants were collected during harvesting on naturally BNYVV infected experimental fields of the Institute of bioenergy crops and sugar beet NAAS of Ukraine in Rivne region. The roots displaying typical for rhyzomania symptoms (stunting, rootlet proliferation, necrosis of vascular bundle, turnip-like shape, root beard) were collected for virus detection. The results of visual diagnostics were confirmed by sandwich ELISA with the use of commercial test systems «LOEWE Biochemica GmbH».

The results of our previous studies that confirmed the presence of BNYVV on beet crops in different regions of Ukraine, and virus harmfulness, determined the urgent need for large-scale breeding work to develop sugar beet hybrids resistant to rhyzomaniya. Given a substantial amount of research to be carried out to achieve this goal it was necessary to develop our own domestic test system. Therefore our further work was focused on the isolation and purification of the virus, followed by rabbit immunization and obtaining antiserum to BNYVV.

Virus extraction was carried out according our own methodology. Roots of virus-infected plants with the highest content of the virus, identified in the previous study, were grinded with fine grater and homogenized in cold 0,01M K-citrate buffer (pH 6) containing EDTA, PMSF and Dioxan, followed by centrifugation about 5000 / min - 20 min. 0,01M citrate-Na and 2% Triton X-100 was added in the resulting material, with than was stirred, incubated in the cold for 1 hour and followed by high speed centrifugation 170000 G for 2 hours at $4^0$C. The precipitate was resuspended in 0,01M citrate-Na buffer, pH 7.5. The purity of viral preparation was measured by spectrophotometry ratio $E_{260}/E_{280}$, where $E_{260}$-absorption coefficient for nucleic acid $E_{280}$ - absorption coefficient for amino acids. The concentration of the purified BNYVV preparation was determined using Edelhoff formula. The virus yield was 10 mg/ml and the purity of virus preparation (1.09) was slightly different from the norm, which is 1.0. Electron microscopy with negative staining revealed the presence of rod-shaped virus particles of different lengths that corresponds to literature data concerning BNYVV morphology.

The resulting purified viral preparation was used for the production of polyclonal antibodies in rabbit. For this purpose the rabbit breeds Chinchilla was immunized with the virus at concentration of 1 mg / ml in three stages at one week intervals. For the first and second doses virus was emulsed with incomplete Freund's adjuvant and injected intramuscularly.

The third dose was injected intravenously. 30 days after the last immunization virus was reinjected subcutaneously at 4 sites along the vertebrae and intravenously. Blood was collected from rabbit marginal ear vein. Indirect ELISA was performed to determine titers polyclonal antibodies. The antibody titer and optimal dilution were shown to be 1:32000 and 1:16000, respectively.

In summary, we received specific to BNYVV antiserum that could be applied in identifying this pathogen, particularly at different stages of plant breeding and further surveys of virus distribution among the industrial crops of sugar beet in Ukraine.

## GENERATION OF SPECIFIC ANTISERUM FOR DETECTION OF PPV

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*Plum pox virus* (PPV) is the pathogen that causes dangerous disease of stone fruit crops and is widespread throughout the world. It attracts great attention of researchers as a quarantine object and causes significant economic losses [2]. The disease caused by PPV was first described in Ukraine in 1966 and since then it has been spreading all over the country. PPV is widespread in almost all regions and is a serious threat to commercial horticulture of our country [1].

The aim of our research was to generate specific antiserum to *Plum pox virus* for its further use in serological virus screening in ELISA [3]. First, the virus was accumulated on herbaceous host plants *Nicotiana bentamiana*. The concentration of PPV in preparation reached 0.6 to 0.8 mg/100 g leaves. Purified preparation of PPV was subsequently used for immunization of laboratory rabbits.

The specificity of the generated anti-PPV antiserum was confirmed by western blot analysis. Titration of this antiserum in ELISA against standard antigens showed its high sensitivity: working dilution of 1:3000 and titer of 1:8000.

As a result of this work, we have generated an antiserum with specificity and sensitivity comparable to those of available commercial test systems. This antiserum proved cost-efficient in routine screening of stone fruit trees for PPV.


1. Budzanivska I.,Usko L., Gospodaryk A., Melnyk M., Polischuk V., 2011. Epidemilology of Sharka Disease in Ukraine. Acta Horticulturae 899: 57-64.
2. Glasa M., 2013. Unfolding the secrets of plum pox virus: from epidemiology to genomics. Acta virologica 57: 217 – 228.
3. Detection and characterization of Plum pox virus: serological methods/ Cambra M. [et al.]. // Bulletion EPPO/EPPO Bulletion. – 2006. – 36 (2). – P. 253-260.


## APPLICATION OF BIOTECHNOLOGIES FOR MICROREPRODUCTION *IN VITRO* OF MINT PEPPERY (*MENTHA PIPERITA L.*) SELECTION SAMPLES


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Mint peppery is a valuable kind in the pharmacological relation of medicinal, essential oil plants of *Lamiaceae* family with a wide application spectrum. Its virus illnesses contamination for today is a principal cause of industrial plantings productivity reduction, because of degeneration and destruction of separate bushes or the whole sites, decrease in quality and quantity of essence oil [1]. The leading place among pathogens is occupied with the viruses, which are leading to plants degeneration, their crop reduction, and further, probably, to utter annihilation of mint peppery plants valuable kinds. For today it is known about 23 viruses which affect plants of genus *Mentha* in the different countries of the world [2, 3]. As mint peppery

is perennial plant, pathogens accumulation and their distribution at vegetative reproduction is occurred in agrocenosises, and that threatens its industrial cultivation [4, 5]. The main condition of virus-free plants reception is healthy uterine plants selection for microreproduction and application of landing material improvement bioprocessing methods, namely apical meristems culture use, thermotherapy and chemotherapy [6].

In Mitrofanova O.V. researches it is shown, that as longer thermotherapy exposition and bigger plants gain are, as it is more probable to receive improved plants apexes [7]. Due to Bugara I.A. recommendations [8] for reception of *M piperita* plants improved from virus infection in nutrient medium structure in conditions in vitro it is possible to add virocide *Ribavirin* (1-β-D-ribofuranozil-1,2,4-triazol-3-karboksamid). But in the literature there is not enough attention is given to improvement and microreproduction of Ukrainian mint peppery selection samples which are the donors of economic-valuable signs and initial forms for industrial grades creation.

Therefore the purpose of our work is optimization of the biotechnological scheme of peppery mint selection samples improved landing material reception with the thermotherapy and chemotherapy application with the subsequent application of a complex of isolated tissues and bodies culture methods in vitro, clonal microreproduction for their genetic potential preservation.

Researches were spent within 2015 in biotechnology laboratory of NUBIP. Mint peppery plants selection samples were without visible signs of the illnesses, they were taken from Research station of medicinal plants of Agroecology and Nature Management Institute, NAAS of Ukraine and were research objects. For each experiment were used 50 explants. At plants-regenerants apical gemmas were separated by means of sterile tools according to standard techniques [6], then they were transferred on modified nutrient medium Murasige and Skug (MS) which was added with 0,75 mg/l of 6-BAP, 0,05 mg/l of kinetine, 0,1 mg/l of adenine, 0,05 mg/l of indoleacetic acid (IAC) and 0,5 mg/l of gibberellins acid (GA).

Explants were cultivated in heat chamber HERAcool-40 of firm HERAEUS at 37 ± 1 °C within 5 weeks, taking into account the previous week of adaptation, so, every day since 25 °C temperature in a heat chamber was increased on 2 °C. Cultivation conditions during the thermotherapy were: temperature was 37 °C, air relative humidity – 90 %, lighting – 3 thousand luxes with the photoperiod 16/8. After thermotherapy received microplants were subcultured on nutrient medium of similar structure with addition of *Ribavirin* (10 mg/l) and cultivated 28 days.

For rhysogenesis was used nutrient medium with half concentration of macro- and the microsalts, containing 0,5 mg/l of IAC and IBA. Plants

adaptation to conditions *in vivo* was spent on substratum: peat:soil universal:pearlite:sand in the ratio 2:1:1:1.

The received plants-regenerants were tested on plants-indicators (*Nicotiana glutinosa L.*, *Nicotiana tabacum L. var. Havana*, *Petunia hybrida Hort*), homogenate was prepared for this purpose from 1 g of vegetative material, then 2 ml of buffer solution was added. Infection was spent mechanically. Plants were cultivated in a hothouse at air temperature 24-25 °C. The results were analyzed on $7^{th}$ and $28^{th}$ day after possible infection. Results processing was realized with mathematical statistics methods use by means of program Microsoft Office Excel 2007.

It is necessary to notice, that during thermotherapy plants growth initiation occurs only on the $11^{th}$-$12^{th}$ day of cultivation. It is noticed, that during thermotherapy the part of plants was lost (table).

**Table** Mint peppery regenerants biometric indices on nutrient medium at thermotherapy ($35^{th}$ day)

| Selection sample | Spear length, sm | Internodes quantity, pcs | Spears quantity, pcs | Plants which were lost, pcs | Prolifera ting plants, pcs |
|---|---|---|---|---|---|
| № M 13-01 | 2,52±0,35 | 6,17±0,84 | 3,02±0,48 | 4 | 92 |
| № M 13-02 | 2,37±0,33 | 5,15±0,98 | 2,83±0,67 | 8 | 84 |
| № M 13-03 | 2,30±0,31 | 4,51±0,98 | 2,79±0,55 | 9 | 82 |
| № M 13-04 | 2,32±0,34 | 4,87±0,95 | 2,94±0,64 | 6 | 88 |

It is established, that during chemotherapy plants had reduced growth rates, had the changed lamina form, therefore biometric indices during this passage were not taken. During the further cultivation on nutrient medium without virocide plants restored morphological signs and differed by intensive growth characteristics, among which it is possible to note reproduction factor at selection sample № M 13-01 which was 1:21, № M 13-02 – 1:16, № M 13-03 – 1:14 and № M 13-04 – 1:11.

Rootaged plants were adapted for conditions in vivo on substratum: peat:soil universal pearlite:sand in the ratio 2:1:1:1. Adaptation lasted for 4 weeks. Engraftment degree has made 98-100 %. By results of biotesting on plants-indicators visually changes were not revealed.

Thermo- and chemotherapy in vitro, as perspective methods of plants improvement, allow to treat simultaneously a considerable quantity of plants in rather small space and do not demand long preparation. The techniques, offered by us, have allowed to receive the landing material deprived of viruses. That was confirmed by the biotests on plants-indicators

and and the method of transmission electron microscopy. Has allowed to receive high reproduction factor on 28th day in the selection samples № M 13-01 – 1:21, № M 13-02 – 1:16, № M 13-03 – 1:14, № M 13-04 – 1:11.


1. Mishchenko L.T., Dunich A.A., Dashchenko A.V., Polishchuk V.P. Viral diseases of medicinal plants // K.,Ptythosociocenter, 2015. – 320 p. *in Ukrainian*
2. Tzanetakis, I. E. Identification, detection and transmission of a new Vitivirus from Mentha / I. E. Tzanetakis, J. D. Postman, R. R. Martin // Arch. Virol. – 2007. – № 152. – P. 2027–2033.
3. Scholthof, K. B. G. Top 10 plant viruses in molecular plant pathology / K. B. G. Scholthof, S. Adkins, H. Czosnek, P. Palukaitis, E. Jacquot, T. Hohn, B. Hohn, K. Saunders, T. Candresse, P. Ahlquist // Molecular Plant Pathology. – 2011. – Vol. 12. – P. 938–954.
4. Species diversity of viruses infecting plants of Mentha genus / Mishchenko L. T., Dunich A. A., Dashchenko A. V., Molchanets O. V. // University proceedings. Volga region. Natural Sciences. – 2014. – № 2 (6). – P. 29–45.
5. Diagnostics of viral diseases of medicinal plants and crop protection system from plant pathogens / L.T. Mishchenko, V.Yu. Maslykov, T.Є. Talankova-Sereda, A.V. Dashchenko, A.A. Dunich, A.L. Boyko // Medicinal plants: perspectives of research materials Third International. Science. Conf. (Berezotocha, 14-15 July 2016) - Kiev, 2016. – p. 110-115
6. Kalinin F. L., Sarnatskaya V. V., Polishchuk V. E. Tissue culture methods in physiology and biochemistry of plants, K: Naukova dumka, 1980. - 488 p.
7. Mitrofanova O.V., Mitrofanova I.V., Lesnikova - Sedoshenkon.P., Ivanova N.N. Using of biotechnological methods for plants improvement and ropagation of virus-free planting material of perspective ornamental plants // Works of the State Nikit. Botan. Gard, V. 138, p. 5 - 56.
8. Bugara I. A. Clonal micropropagation and recovery of *Mentha piperita* L. *in vitro*. *Scientific Notes of Taurida V.I. Vernadsky National University. Series: Biology, chemistry*, *26* (1), p. 10–15.

# DETECTION OF COMMON AND NECROTIC ISOLATES OF *POTATO VIRUS Y* IN POTATO SEED

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*Potato virus Y* (PVY) is one of the most economically important plant pathogens, often listed in the top five viruses affecting both yield and quality in field-grown vegetables. Currently, there are nine groups of PVY strains, which differ in biological, serological and molecular properties [1]. Symptoms of lesions associated with PVY infected plants, vary depending on the strain of virus and potato variety and range from no symptoms and mild local lesions and common mosaic to rugose mosaic, systemic necrosis and plant death. This makes it difficult to diagnose the virus field for the certification of seed and leads to a significant decrease in quality seed potatoes.

In the world for a long time as the dominant were considered populations $PVY^{O}$ – common strains, but recently he replaced necrotic strains $PVY^{N}$, $PVY^{NTN}$ and other strains that are as recombinant strains between groups, and new strains arising from mutations [3]. Thus, the study of populations of strains PVY needed to improve diagnosis of the virus in potato plantings and maintenance of breeding programs to create varieties resistant to PVY.

Single study conducted on the circulation common and necrotic strains PVY in Ukraine [4].

Potato tubers have been collected in the harvest period and were treated accordingly for breaking of dormancy. Plants grown from tubers in the conditions cultivation room in artificial light with 16 hour light period at a temperature of 21-24° C. 30 days after germination plants visual assessment conducted and tested for virus content.

The detection of viruses has been performed by ELISA (double sandwich option, DAS-ELISA), with the help of commercial test systems - LOEWE, Germany. The results have been recorded at wavelengths of

405/630 nm. Data processing of optical density of samples has been performed by means of descriptive statistics, determining the average and standard deviation data. The threshold optical density, which distinguishes the positive results of an enzymatic reaction on the value of the background, has been determined for each plate individually as it is recommended (Technical information). Morphology of viral particles in preparation of potato saps has been investigated by means of transmission electron microscopy (JEM 1230 microscope (JEOL, Japan) using negative contrast agents of 2% solution of phosphoric-tungsten acid and 2% solution uracil acetate within 2 min [5]. We used plants-indicators *N.bentamiana* and *N. tabacum*, cv. Samsun for biological testing aged 4-6 leaves.

Plants indicator was inoculated by infected potato sap, containing PVY and without other viruses. One part of plants-indicators were inoculated by sap plants potato variety Slovyanka, which were revealed necrosis of veins, another party – sap of Lady Rosetta plants with symptoms light mottling. 9 days after inoculation sap of potato plants Lady Rosetta, the first symptoms of viral infection on plants *N. bentamiana* and *N. tabacum*, cv. Samsun appeared as a clarification of the veins. Further systemic veins necrosis developed, sometimes the plants were died through necrosis veins. In infected tobacco plants were found filamentous particles similar in morphology of PVY by transmission electron microscopy (Fig.1). Data ELISA and RT-PCR from samples indicator plant confirmed the contents of PVY. Thus, isolate from potato plants, which we called 6-Pot, apparently belonging to the necrotic strain of PVY.

In tobacco plants, inoculated with isolate of potato varieties Slovyanka, visible symptoms of infection were not found. However, data ELISA and RT-PCR confirmed the contents of PVY in these plants. Filamentous virus particles, similar in morphology of PVY, were also found in purified preparations of these plants. Thus, this isolate, named our 19-Pot, apparently, belongs to a group of ordinary strains of PVY.

In addition, the definition of phylogenetic relations Ukrainian isolate PVY [6], shows that it's an recombinant virus with possible phenotypic manifestations as ordinary strain O and necrotic strain N, which will depends on the specific conditions of viral infection. The authors also claim that they discovered isolate PVY strains belonging to group O, for more detail N:O, in the recombinant between groups N and O; in another classification of isolates - to ON-WilgaN:O. These reasons encouraged to conduct in-depth research PVY population in Ukraine to find effective measures to control this dangerous pathogen. In determination of the interaction of the virus is important combination of different approaches that will contribute to a complete understanding of the interactions between host plants - PVY. Knowledge from biochemical, transcriptomic,

proteomic, metabolomic, and phenomic studies would contribute to the construction of a model of potato response to PVY infection.


1. Response of potato cultivars to five isolates belonging to four strains of Potato virus Y/ B.Nie, M.Singh, A.Murphy et al. // Plant Diseases. – 2012. – V. 96. – P.1422–1429.
2. Potato virus Y: a century of evolution/ A. Blanchard, M. Rolland, C. Lacroix et al. // Current Topics in Virology. – 2008. – V.7. – P. 21-32.
3. Genetic diversity of the ordinary strain of Potato virus Y (PVY) and origin of recombinant PVY strains / A.V. Karasev, X. Hu, C.J. Brown, C. Kerlan // Phytopathology. – 2011. – V.101. – P. 778–785.
4. Shevchenko O.P. Distribution and diagnosis of necrotic strain of Potato virus Y in Polissya Ukraine [Text] / O.P. Shevchenko // Bulletin KhAI: Coll. Science. Hark works. nat. Agrar. Univ / Hark. nat. Agrar. Univ. - X: KhAI, 2006. - № 5. - P. 110-115.
5. Salyha, Iu. T. – Snitynskyi, V.V. 1999. Electron microscopy of biological objects – Lviv. 152 p.
6. Budzanivs`ka, I.G. – Polishhuk, V.P. 2015. Phylogenetic analysis of RNA plant virus circulating in Ukraine. Available at: http://virology.com.ua/wp-content/uploads/2015/02/Budzanivska_Polischuk_Last.pdf


# EVALUATION OF RESISTANCE PLANTS *NICOTIANA TABACUM*, L. AND *NICOTIANA RUSTICA* L. TO NECROTIC AND COMMON STRAINS *POTATO VIRUS Y*


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*Potato virus Y* (PVY) – the member of the genus *Potyvirus* family *Potyviridae*, that can infect potato, tomato, tobacco, pepper and other plants of the *Solanaceae* family. Determination of strains based on the symptoms

that occur when host plants, potatoes and tobacco, as well as serological reactions of selected isolates.

Currently, about nine groups detected strains, but the most basic and common until recently considered are three groups of strains: PVY-N, PVY-O and PVY-C [1]. Isolates belong to group of PVY-N cause severe symptoms necrosis of veins in the tobacco plant *Nicotiana tabacum*, and in the field can lead to a significant deterioration in the quality of products of this culture. At the global level, *Potato virus Y* is certainly the most damaging virus of tobacco. The latest survey of CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) on tobacco viruses confirms this situation. Losses due to necrotic strains of the virus are increasing in many countries. The impact of this virus on Burley and Virginia tobacco are reduction of size and weight of leaves, plant height, and yield. The earlier the infections occur, the greater the impact on the tobacco crop. In Chile and New Zealand, heavily infected crops have experienced yield reductions exceeding 70%. The chemical quality of harvested tobacco is reduced also in the presence of PVY. An increase of nicotine, nornicotine, total nitrogen, nitrogen-insoluble acid and nitrate content can be observed [2].

To meet the needs of modern plant breeding should guide the work to identify variety group with stable resistance in the face of fierce natural and artificial infectious background to get the expected effect of the source resistance. Great importance is raising new varieties of group stability. For successful breeding in this area required starting material with resistance group to defeat pathogens that would fully meet the increasing demands on breeding performance and high level of adaptability to adverse environmental factors. Therefore, the study of varieties with a view to providing new sources of resistance to major pathogens remains relevant and necessary [3].

In our studies we were evaluated of resistance plants *N.tabacum* varieties Samsun 155, Sobalchsky 34/40, Sobalchsky 193 and *N.rustica*, varieties Matsui Field and Actec. Plants were inoculated of potato plants' sap, which were discovered PVY isolates belonging to group strains: isolate of $PVY^N$ and isolate of $PVY^O$. Belonging of isolates to a group of strains determine by biological testing on plant indicators. Plant indicators and plants *N.tabacum* and *N. rustica* were grows in greenhouse condition at 25 єC and 16 light period. We used plants *N.tabacum* and *N. rustica* for testing aged 4-6 leaves. The five plants per cultivar were inoculated with the viral inoculums. The control plants were inoculated with PBS (pH 7.4). Three week later plant leaves were detected by DAS-ELISA and transmission electron microscopy.

Viruses determined by DAS-ELISA using of commercial test systems LOEWE, Germany. The results have been recorded at wavelengths of 405/630 nm. Data processing of optical density of samples has been performed by means of descriptive statistics, determining the average and standard deviation data. The threshold optical density, which distinguishes the positive results of the enzymatic reaction on the value of the background, has been determined for each plate individually as it is recommended (Technical information). Morphology of viral particles in preparation of potato saps has been investigated by means of transmission electron microscopy (JEM 1230 microscope (JEOL, Japan) using negative contrast agents of 2% solution of phosphoric-tungsten acid and 2% solution uracil acetate within 2 min [4].

The leaves of plants *N.tabacum* cv. Samsun 155 and cv. Sobalchsky 34/40 inoculated isolate $PVY^N$, showed necrosis veins that spread systemically in the plant.

Plants inoculated isolate $PVY^O$ did not show any symptoms or only mild mosaic symptoms. According to ELISA and electron microscopy believe accumulate in plants varieties *N.tabacum,* cv. Samsun 155 and cv. Sobalchsky 34/40, but in plants *N.tabacum,* cv. Sobalchsky 193 and plants *N.rustica* viruses not found. The evaluation results are shown in table 1.

**Table1.** Results of inoculation tobacco plants by necrotic and ordinary PVY isolates

| Plants/variety | PVY isolates | |
|---|---|---|
| | $PVY^N$ | $PVY^O$ |
| *N.tabacum,* cv. Samsun 155 | NV, M | MM |
| *N.tabacum,* cv. Sobalchsky 34/40 | NV, M | No symptoms |
| *N.tabacum,* cv. Sobalchsky 193 | No symptoms | No symptoms |
| *N.rustica,* cv. Matsui Field | No symptoms | No symptoms |
| *N.rustica,* cv. Actec | No symptoms | No symptoms |

NV – necrosis veins, M – mosaic, LM – mild mosaic

Thus, the results of laboratory analysis, varieties *N.tabacum,* cv. Sobalchsky 193 and *N.rustica,* cv. Matsui Field and cv. Actec can be resistant to necrotic virus strain. Is needed more research in the field to confirm our results revealed tobacco plant resistance to necrotic isolates circulate in Ukraine.

1. The naming of Potato virus Y strains infecting potato / R. P. Singh, J. P. T.Valkonen, S. M.Gray et all.// Arch. Virol. – 2008. – V.153. – P.1-13.

2. Virus des solanacées: du génome viral à la protection des cultures /G. Marchoux, P.Gognalons, K.G.Sélassié. – Versailles: Quae, 2008. – 843 p.
3. Savina O. I. Evaluation of basic collection on stability against tobacco diseases [Elektronny`j resurs] / O. I. Savina // Agrobiologiya. - 2013. - V.10. - P. 53-57. - Rezhy`m dostupu: http://nbuv.gov.ua/UJRN/agr_2013_10_15
4. Salyha Iu. T., Snitynskyi V.V. Electron microscopy of biological objects – Lviv, 1999. – 152 p.

# PHYLOGENETIC ANALYSIS OF DIFFERENT GENOME REGIONS OF NEW ISOLATES OF CUCUMBER MOSAIC VIRUS FROM UKRAINE

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Since its discovery, *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) has been found in all parts of the world, and numerous strains have been characterized. In our previous projects we've obtained several partial sequences of coat protein gene. Isolates from subgroups IA and IB were detected in different regions on plants from *Solanaceae* and *Cucurbitaceae* family [1]. Although phylogenetic analysis of Ukrainian isolates was done previously on the basis of the coat protein sequences, here we extended this analysis to the 2b and movement protein sequences of isolates from different hosts and locations.

The purpose of current study was to obtain and analyze the nucleotide sequences of coat protein (CP), movement protein (MP) and 2b genes.

Plant samples were collected in different regions during 2015 year. Detection of viral antigens was performed by DAS-ELISA using commercial test systems of Loewe (Germany). For further analysis we randomly selected three samples shared typical symptoms of CMV (yellowing, mosaics, mottling, fruit deformation, leaf distortion, stunting),

positive results in ELISA and distinct origin (Lviv (Western Ukraine), Poltava and Cherkasy regions (Central Ukraine)).

Total RNA was extracted from plant samples using RNeasy Plant Mini kit (Qiagen, UK). Reverse transcription polymerase chain reaction was accomplished using specific primers to CP, MP, 2b gene of CMV (expected product size – 500 bp, 800 bp, 300 bp respectively) [2, 3]. Then obtained amplicons were purified and sequenced using Applied Biosystems 3730x1 DNA Analyzer with Big Dye terminators, version 3.1 (Applied Biosystems, USA). Phylogenetic analysis was conducted using Neighbor-Joining method in MEGA 6.

According to the topology of phylogenetic tree based on CP and MP gene regions, detected isolates belong to subgroup IB, which had been originated from East Asia. The homology level of CP between obtained isolates and previously reported in Ukraine was high (98-100%). The sequences of MP gene were also highly homologous (97-99%).

Expectedly, phylogenetic trees interfering the relationships between different isolates based on CP and MP regions shared similar topologies; however, the topology of phylogenetic tree based on 2b sequences was different. Generally, the evolution rate of the 2b gene is known to differ from the other genomic regions [4].

The homology level of nucleotide and amino acid sequences 2b gene region ranged from 95 to 99% and from 93 to 100% respectively. We found that similarity of isolates CMV-28 and CMV-58 are considerably higher (up to 100%). These two isolates are extracted from squash plants (*Cucurbita pepo L.*). The third isolate was extracted from cucumber (*Cucumis sativus L.*). The homology between CMV-36 and other obtained isolates was 95% and 93% at nucleotide and amino acid levels. Unique amino acids were found in several positions: Isoleucine at 42 position of CMV-28 and CMV-58, Leucine at 63 position of CMV-36. Since these isolates have different host origin, we suggested that correlation between hosts and amino acid substitutions may be present. However, phylogenetic tree based on amino acid sequences revealed no significant correlation.

1. The first evidence of subgroup IB isolates of Cucumber mosaic virus in Ukraine / T.P. Shevchenko [et al.]. // Biopolymers and Cell. – 2015. – Vol. 31, №1. - P. 57–62.
2. Biological and Molecular Characterization of Cucumber mosaic virus Isolate Causing Shoestring Disease of Tomato in India which has Closer Affinity to European or East Asian Isolates of CMV / D. Pratap [et al.]. // Indian J. Virol. – 2012. – Vol. 23, №1. – P. 57–63.

3. Ahmed A.A. Double infection with Cucumber mosaic virus and Plum pox virus (sharka) in apricot trees / A.A. Ahmed, Fath-Allah M.M. // International journal of virology – 2012. – Vol.8, №1. – P.50-60.
4. Molecular variability of five Cucumber mosaic virus isolates from china / Y. Liu [et al.]. // Acta Virol. – 2009. – Vol. 53. – P. 89−97.

# THE DIAGNOSTICS OF POTATO DISEASES, CAUSED BY POTATO VIRUS Y

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The potato is one of the main agricultural crops in Ukraine, which is an indispensable food, widely used for animal husbandry and as a raw material for the processing industry. Among the large number of diseases on this crop a viral infection are a significant factor in reducing the productivity and quality of potato tubers.

In Europe celebrated the spread of *Potato virus Y*, especially necrotic strains, a significant harmfulness decree also research Belorussia, and Russia.

Our studies have been conducted in the branch of virology of the Institute of Agricultural Microbiology and Agroindustrial Productions. We have used a set of diagnostic methods: symptomatology, imunolog, Electron Microscopy to identify viruses. For the biological testing we used indicator plants such as: *Nicandra physaloides* L., *Nicotiana tabacum* L., *Nicotiana rustica* L., *Nicotiana oscidentalis* L., *Physalis floridana* L., *Lycopersicum esculentum* L. (Mill.).

It has been determined that in Ukraine spread of Potato virus Y is significantly increased and the range of infected with this virus sorts is expanded. For the finish time in Ukraine quite a new potato disease resulting in necrotic rings straining bulbs has been found, the main pathogen being *Potato virus Y* (necrotic strains, $PVY^{NTN}$).

The analysis of the tested isolates taken from the plants of various potato sorts, shows the predominance of necrotic strains (80 % of the

samples tested). On the basis of studying strains of the virus peculiarities of diagnosing potato diseases have been found. Necrotic and common strains of *Potato virus Y* have been selected for further use in research and practice.

It has been determined that potato plants affected by Necrotic strains of *Potato virus Y* reduce productivity from 11,1 to 62,3 %.

Also, susceptible to *Potato virus Y* sorts grown in Ukraine have been found.

The data obtained are taken into consideration for improving the methods of checking *Potato virus Y* in growing seed potatoes and monitoring virus diseases of plant.

## DETECTION AND IDENTIFICATION OF BLACK AND RED CURRANT VIRUSES IN THE SYSTEM OF PLANTING MATERIAL PRODUCTION

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Currants are susceptible to a range of viral infections, some of them can cause complete loss of yield and, conse-quently, lead to great material losses of farmers. According to European Plant Protection Organization the most harmful are *Blackcurrant reversion virus* (Nepovirus, BRV), *Strawberry latent ringspot virus* (Sadwavirus, SLRV), *Raspberry ringspot virus* (Nepovirus, RpRSV), *Arabis mosaic virus* (Nepovirus, ArMV), *Cucumber mosaic virus* (Cucumovirus, CMV) and *Gooseberry vein banding associated virus* (Badnavirus, GVBaV) [1]. Development and implementation of rapid diagnostic methods such as ELISA and PCR allows to provide early detection of these viruses in plantations and to prevent their spread by vegetative propagation of planting materials and vectors.

During the 2012-2016 selection of virus-free clones of black and red currant varieties was conducted in the Department of virology, plant health and propagation of fruit and berry cultures. Samples were selected without visual symptoms of virus infection. For detection of viruses by ELISA LOEWE Phytodiagnostics commercial certified kits were used for identification of SLRV, RpRSV and ArMV. Reverse transcription-PCR was

used for detection of BRV by using a pair of primers BRV1-10F/10R [2]. For identification of serotypes I and II of CMV multiplex PCR was held with primers CMV I (F)-I (R) and CMV II (F)-II (R) respectively [3]. Extraction of RNA was performed by commercial set MasterPure™ Complete DNA and RNA Purification Kit (Epicentre, USA) according to manufacturer's recommenda-tions. For RT-PCR SuperScript® III One-Step RT-PCR System with Platinum® Taq DNA Polymerase (Invitrogene) was used according to the manufacturer's instructions.

More than 40 varieties and perspective hybrid forms of currants has been tested. Totally 30% of tested material was infected. The incidence of SLRV prevailed (20,6%). More than half of infected plants had complex infection. The most common was infection by RpRSV and SLRV. Only sporadic cases of infection with ArMV were detected and one sample was infected with blackcurrant reversion virus. There were no samples found infected with CMV. As a result the database of virus-free plants, that meet international requirements for prebasic motherplants, was created.


1. OEPP/EPPO (2008) EPPO Standards PM 4/9 (2). Certification scheme for Ribes. EPPO Bulletin. Vol. 38, Is. 1: 14–18.
2. Dolan A., MacFalane S.A., McGavin W.J. et al. Blackcurrant reversion virus: Validation of an improved diagnostic test, acceleration testing in breeding and certification of blackcurrants. Journal of Berry Research
3. Chen Sh., Gu H., Wang X. et al. Multiplex RT-PCR detection of Cucumber mosaic virus subgroups and Tobamoviruses infecting Tomato using 18S rRNA as an internal control. Acta biochim Biophys Sin 2011, Vol. 43, Is. 6: 465-471.


## ABOUT VIRUSES IN THE SPHERE OF HYTOSANITARY RATIONING OF POTATO SEED QUALITIES


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Potato viruses are numerous and the diseases caused by them are various: from mosaic type infections to yellows type.

To form seed market with high varietal and seed qualities in Belarus great attention is paid to viral infection control. In the acting national standard the phytosanitary rates are determined for viruses X (*Potato virus X*), S (*Potato virus S*), M (*Potato virus M*), Y (*Potato virus Y*), L (*Potato leafroll virus*), A (*Potato virus A*) in the latent form, soil viruses – rattle (*Tobacco rattle virus*) and mop-top (*Potato mop-top virus*). At phytovirusological control the plants infection by light (common mosaic, mosaic leafroll) and severe virus diseases (rugose mosaic of potatoes, streak mosaic, leaf-rolling) in different seed potato categories (initial material, original, elite and reproductive seeds) is observed [1].

Meanwhile, the external virus infection depending on the disease agent specific belonging can be manifested on growing potato plants, not only by indicated above symptoms, but in the form of yellow spot, folding mosaic, aucuba mosaic, stem variegation, paniculate top, curly dwarfness and etc.

Recently during potato vegetation yellow leaf spot is discovered. Such symptoms manifestation is registered on foreign and local selection varieties. The infected plants are usually distributed along all the massive of plantings as a witness of infection transfer with seed tubers. One should point out that based on different data, the symptoms of virus infection in the form of yellow spot are characteristic for aucuba mosaic virus (*Potato aucuba mosaic virus*) [2, 3, 4], rattle virus [4], mop-top virus [3, 5].

In our researches (2014) the plants of foreign selection cv Aureya with the yellow spot symptoms were checked for virusological control for mosaic X, Y, S, M group of viruses. Their ratio in plants with the positive reaction on virus infection was distributed in the following order: complex of viruses Y+S+M - in 33,3 % cases, S+M – 13,3 %, Y+S – 6,7 %, Y+M – 6,7 %, single infection Y – 13,3 %, M - 26,7 %. Potato virus X was not revealed at all. In plants of foreign selection cv Pirol with the yellow spot symptoms also the viruses Y, S and M were detected.

The same conformity to single, two and three-component of Y, S, M viruses infection was observed in plants of local cv Lileya elite seeds category with the single type yellow spot disease symptoms. For this, also prevailed three-component virus infection Y+S+M (55,5 %). A share of two-component S+M, Y+S, Y+M and single M virus infection was less (each a little more than 11,0 %).

Among the ethiological yellow spot disease agents, as it is above-stated, there are rarely met earlier in potato in Belarus viruses such as rattle, mop-top. Using the laboratory diagnostics abilities, at present rattle virus distribution, for example, in Gomel district is determined at the level of 9,0-19,0 %, whereas, plantings infection by mop-top virus is of restricted character [6].

By phytovirusological situation study in cv Lileya (2015) growing plants on different nutritive backgrounds a prevalent manifestation of yellow spot disease (25,0 %) was revealed in a variant without fertilizers. Its distribution was 2,2 times lower (11,2 %) against a background of organic fertilizer (manure, 60 t/ha). In a minimum quantity (6,2 %) such symptoms were detected against a background of organic–mineral fertilizers system (manure + NPK). On the one hand, such a phenomenon can be explained by a possibility of tested virus infection "masking", on the other hand, by nutrition conditions influence as a factor of external influence on a plant-virus system integrity affecting either a degree of potato plants infection or pathogenic peculiarities of the disease agent. One should point out that the balanced rates of fertilizers application is an integral technological demand for seed potato growing in Belarus.

As a result of virus diseases "masking" it is not always possible to get a real idea on varietal qualities of potato plantings. In particular, the phytosanitary norms both for viroses and yellow spot disease infection in the acting standard for seed potato are absent. In this case a significant distribution of a similar type viroses increases a risk of late detection of etiological disease agents for correction the measures directed to phytovirological situation management in the production of high quality potato tubers.

1. Kartofel semennoy. Tekhnicheskie usloviya: STB 1224-2000. - Vved. 01.07.00. - Minsk: Gosstandart, 2000. - 13 s.

2. Ambrosov, A.L. Virusnye bolezni kartofelya i mery borby s nimi / A.L. Ambrosov. - Mn., 1975. - 208 s.

3. Blotskaya, Zh.V. Virusnye, viroidnye i phytoplazmennye bolezni kartofelya / Zh.V. Blotskaya. - Minsk: Tekhnalogiya, 2000. - 120 s.

4. Soroka, S.V. Virusy i virusnye bolezni selskokhozyaistvennykh kultur / S.V. Soroka, Zh.V. Blotskaya, V.V. Vabishchevich : nauch. red. R.V.Gnutova. - Nesvizh: Nesvizh. Ukrupn. Tip., 2009. - 128 s.

5. Virusy i virusopodobnye bolezni i semenovodstvo kartofelya / Pod red. G. Lebenshteyna, F.Kh. Bergera, A.A. Branta, R. Kh. Lousona; otv. za vypusk E.V. Truskinov. - 2005. - 283 s.

[6. Rusetsky, N.V. Monitoring tobacco rattle virus, potato mop-top virus i alfalfa mosaic virus v posadkah kartofelya Gomelskoy oblasti / N.V. Rusetsky, V.A. Kozlov, A.V. Chashinsky // Kartofelevodstvo: sb. nauch. tr. / RUP "Nauch.-prakt. Centr NAN Belarusi po kartofelevodstvu i plodoovoshchevodstvu"; redkol.: S.A.Turko (gl. red.) [i dr.].- Minsk, 2014. - T. 22. - S.139-149.

# SESSION "GENERAL, MOLECULAR VIROLOGY AND VIRUSES OF MICROORGANISMS”

# INTERACTION BETWEEN PHAGES AND BACTERIA AS A TOOL FOR THE OBTAINING OF IMAGES

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Creation of images by controllable culturing of microorganisms in certain patterns (microbial art) now became a very special branch of skill at the interface between science and art [1]. Usually it can be performed by the application of the microorganisms with the intensively colored colonies [2]. Another approach is based on the using of transgenic bacteria expressing fluorescent protein genes [3]. In both cases the stiff nutrient medium (agar) is used as a background, where the image can be formed as the result of the growth of the bacterial colonies, thus bacteria appear as a tool. However inverse approach, where bacterial lawn serves as the background and the image is formed by the lytic action of the virus (bacteriophage), could be applied not only for artistic aims but for the practical use. Thereby the aim of this work was to demonstrate a possibility to obtain an image on the bacterial lawn as a consequence of the interaction between bacteriophage and bacteria.

The bacterial lawn was obtained by the standard method using the 1,5% agar with the nutrient medium and the 0,7% agar containing Escherichia coli culture. Stencils with the preparation of the bacteriophage T4 were applied. Samples were incubated during the twenty-four hours at +37°C. After that stencils were removed and the samples were stained by Coomassie blue R-250 or fuchsine (with further fixation by the 7% acetic acid).

Several approaches to obtain the image by the interaction between bacteriophage and bacteria were applied. It was demonstrated, that the use of filter paper stensil allows to obtain more accurate and controllable images, than the use of the printing paper stensil. The possibility of the reversed stencil use (where the image is formed not by the lytic zone but by

the zone of bacterial growth) was demonstrated as well. Also the possibility of the partial staining of the obtained image was explored. It gives an opportunity to obtain polychrome images using available colorants. Summarizing the above it should be noted, that this approach could be used not only for the artistic aims but as well for the practical use, for example, for the restriction of the action of microorganisms in out-of-the-way places.


1. Torrice M. (2009) Petri dish artists. *Science* (326) p.777.
2. http://www.microbeworld.org/component/jlibrary/?view=article&id=14215
3. http://www.tsienlab.ucsd.edu/Images.htm


## DETECTION OF NEW PHAGES IN THE HETEROGENEOUS POPULATION OF *ERWINIA AMYLOVORA* BY METHODS OF ELECTRON MICROSCOPY AND RESTRICTION ANALYSIS


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The current data proves that all bacteriophages, which persist with important Erwinia amylovora virulent pathogenic bacteria, are not always active on the main pathogen. The reason for this phenomenon remains unknown. It is likely that the main pathogen phages are minor part of the phage isolate and innovative approaches are needed for their selection, including the usage of Pantoea agglomerans as a universal indicator system.

Known phages isolated from specified ecological niches have different morphology and primary sequence of the genome. These phages are also polyvalent and affect P. agglomerans, which usually accompanies the pathogenic erwinia.

In the submitted paper the results of studies related to the heterogeneity in the bacteriophage population of phytopatho-genic bacteria, that cause fireblight on woody fruit plants in ecological niches, are discussed for the first time. This phenomenon creates significant losses in agriculture, including horticulture.

The conventional biological tests of identifying bacteria and their phages were involved in studies. To determine the molecular biological characteristics of phages were used: electron microscopy, DNA electrophoretic studies, restriction and chromatographic methods.

It was proved that isolates include phage population represented by several phage variants that differ in morphological and structural parameters, molecular weight and restriction pattern.

These phage variants are characterized by different patterns of HpaI - restriction. Electron microscopy is conducted with a common contrast method with 2% uranyl acetate and 2% phospho tungstic acid.

It is shown that phage isolates have unique biological properties. One of them is found in dynamics in terms of its selection manufacturing process. And the other is distinguished by its lability during chromatographic separation, it is unstable after purification in CsCl gradient and detection is successful only when operating it on phage-stable bacterial mutants.

The technique of these phages separation for obtaining phage variants clean lines, which enables further usage of these viruses for different technological processes, is submitted in the paper. Detection of new viruses will more fully describe their diversity and presence in the relevant isolates and to explore the biodiversity and ecology.

The obtained results are important for such industry as agroindustrial manufacturing and provide the foundations for a comprehensive fight against diseases of woody plants.

# INCORRECT USAGE OF THE TERM “SPECIES” IN THE TAXONOMY OF VIRUSES (DISCUSSION)

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Important questions about the position of "species" in modern taxonomy of viruses of different organisms are considered. The key properties of viruses based on their evolution, genetic and molecular structure, ecology, variability and intracellular functions are discussed. The assessment of viruses as special messengers of nature that do not meet the classical canons of species (taxon) in biology suggested by C. Linnaeus (1735) is given. For that time he successfully suggested a binary approach (from the latin. *binaries* –- double) and defined it as universal and discrete. Later Ch.Darwin considered the species for different organisms from the standpoint of evolutionary concepts.

Based on different families of viruses an unmatched position of "species" as a taxonomic unit is considered. Starting from 1962 Andrй Lwoff, Robert Horne, and Paul Tournier offered certain principles in virus classification using Linnaean system. Later D.Baltimore put some positions of the mechanisms of mRNA formation (several major groups) into the classification of viruses. However, specialists of many directions in biology do not accept "species" as taxon from general biological positions. Due to acellular system of viruses and their molecular genetic status, variability, they do not correspond to the classic definition of species [Boyko A.L., 2003].

Based on analysis of the viroids, prions, bacteria, phytoplasm "nanobacteria" properties in the submitted work position of classifying them by some researchers as "viruses" is criticized. In the materials some positions of epigenetics and role of the science of viruses in this chain are discussed [P. Shpork, 2013]. Thus, using modern taxonomy of viruses (ICTV) [Boyko A.L. et al, 2014; A.King. et al, 2015]: order (*-virales*); family (*-viridae*); subfamily (*-virinae*); genus (*-virus*); species (*virus*) it is possible, for example, to replace term **“**species**”** into *virgen* word (viral gene) providing possibilities to accept the classification of viruses for today period of scientific achievements in the biological field more reasonably.

1. Бойко А.Л. Основи екології та біофізики вірусів. – К. фітосоціоцентр, 2003. – 167с.

2. Петер Шпорк. Читая между строк ДНК. – М. «Ломоносовъ», 2013. – 272с.

3. Boyko A.L., Zarytskyi N.N., Demchenko A.A., Polischuk V.P., Yuzvenko L.V., Lazarenko L. N., Babenko L. P. Spread and morphological-structural properties of plant rhabdoviruses and similar phathogens in Basidiomycetes. – Microbiologichny zhurnal v.76, №2, 2014. – p.41-46

4. Virus taxonomy. Ninth of the International Committee on Taxonomy of viruses. Ed. Andrew King et al., Wien: Springer-Verlag, 2012.- 1327 p.

## PREZENT AND FUTURE OF THE BIOLOGICAL WEAPONS CONVENTION

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The Biological and Toxin Weapons Convention (BTWC) was the first multilateral disarmament treaty to ban the production and use of an entire category of weapons. It entered into force on 26 March 1975. Over the years, increasing numbers of States joined the Convention. There are currently 175 States Parties. The BTWC effectively prohibits the development, production, acquisition, transfer, stockpiling and use of biological and toxin weapons and is a key element in the international community's efforts to address the proliferation of weapons of mass destruction.

According to D.Gerstein [1], the most important characteristics of the Biological Weapons Convention are as follow. It:

1) eliminates an entire class of dangerous weapons;
2) provides an unequivocal norm against the use of biological weapons;
3) provides an international forum for dialogue concerning biological defense issues;
4) has an important economic dimension;
5) is gaining more importance as the spectrum of potential biological threats grows;

6) provides a forum for coordinating preparedness and response capabilities against a spectrum of biological threats;
7) is an arms control agreement that relates directly to public health, the environment, food security, and biodiversity;
8) provides direct linkages to international security mechanisms;
9) relates to dual-use capabilities in a way that no other arms control treaty does;
10) has responsibilities for implementation starting from international organisations down to the individual.

Although the BTWC sets a high standard of international conduct, it is presently of limited practical effectiveness because it lacks verification mechanism and universal adherence, while relying on a voluntary confidence-building measures.

Originally the BTWC was considered through the focus of attention to the states offensive programs, however the perception of the biological threat has shifted from states developing a biological weapons capabilities to use against other states to naturally occurring infectious disease, misuse of technology, accidents, etc. [2]. The complexity of the contemporary threats is greater than during previous historical periods. The range of biological agents that could be used as weapons is expanding, and can include a broad range of biological incidents from naturally occurring pandemics such as influenza to the state use of BW.

The existing confidence-building measures within BTWC are useful but not sufficient for ensuring the efficient implementation of the Convention and the safety guarantees. Today, the biological terrorism, natural and technologically induced emergency situations caused by the release of biological hazardous agents represent a real threat to our planet. The Ebola outbreak, which caused more than dramatic consequences for the whole humanity, mobilized the international efforts to overcome this dangerous disease. At the same time the world continues to face a number of possible biothreats of natural and artificial origin. In particular, those arising from the new emerging and reemerging dangerous infectious diseases, drug-resistant diseases, threats arising from the progress in modern life sciences, from the expanding army of “do it yourself” or “garage” bio-engineers. Unfortunately, examples of most dangerous pathogens escape even from the most biosecurity sophisticated laboratories are known. So the life sciences progress also creates possibilities for intentional and/or deliberate misuse of dual-use materials, technologies and knowledge.

It is also worth to mention that the Biological Weapons Convention does not exist in vacuum. What we can see during the past decade is the proliferation of initiatives that relate to biological weapons and naturally occurring infectious diseases. This is a multidimensional process, and

includes international level, state level, the level of NGO, public-private partnerships and so on. However, the proliferation of initiatives, actors, and financial resources in global health during the past decade has reached a point where the proliferation may harm efforts to improve possibility to control biothreats [2].

What we are missing in all of this is the "umbrella concept", which would combine all the initiatives, coming either from the state governments, academia or non-governmental sector. And the integrative task is critical. To move forward we have to recognise that biosecurity policy requires globalised governance of biosecurity threats. To achieve sustainable biosecurity in the twenty-first century we need to build globalised governance mechanisms. The format and scope of that is the issue for discussion. However the response to the current challenges to the biological weapons ban must be network-based, enabling multiple actors with different mandates [3].

The Eight BTWC Review Conference to be held in November 2016 will be an important opportunity to engage in a dialogue on the functioning of the Convention and on ways to improve it. At the Eight Review Conference the international community has to address the complicated issues that demand urgent attention. What is the expectation regarding the future of the BTWC? What is the growing spectrum of potential biological threats? How we can balance the progress in the life sciences and the need for sufficient security? And the last but not least, how should look like the international architecture of globalised biosecurity governance?


1. Gerstein D. National Security and Arms Control in the Age of Biotechnology: the Biological and Toxin Weapons Convention. Rowman and Littlefield Publishers, Inc., 2013 - 234 p.
2. Fidler D., Gostin L. Biosecurity in the Global Age: Biological Weapons, Public Health, and the Rule of Law. Stanford University Press, 2008 – 306 p.
3. Beyond the BTWC RevCon. Disarmament Forum, 2011, no.1 – 72 p. http://www.unidir.org/files/publications/pdfs/beyond-the-btwc-rev-con-en-313.pdf

# CONSERVED NON-CODING ELEMENT OF BACULOVIRUS GENOME IS ABSOLUTELY REQUIRED FOR THE TRANSPORT OF NUCLEOCAPSID TO THE NUCLEAR PERIPHERY

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Baculoviruses comprise a diverse group of large enveloped double stranded DNA viruses that infect invertebrates. Baculoviruses appear to be a tool for a wide range of practical applications: they are used traditionally as environment-friendly insecticides, their recombinant derivatives are widely used as expression vectors for recombinant protein production and for vaccine manufacturing, they are also considered to be prospective vectors for gene therapy. Baculovirus molecular biology appears to be a well studied field, however, there remain critical unanswered questions: the model of baculovirus replication, the mechanisms of DNA cleavage and packaging as well as the mechanism of baculovirus lathency have not been suggested yet. As it is assumed, by the analogy with other viruses, that all the processes listed above may be mediated by the activity of some DNA non-coding elements (replication origins (oris), cleavage/packaging signals, latent ori/integration signals respectively), the identification of non-coding functional elements in the baculovirus genomes can be regarded as a focal task on the way of investigation of these processes.

Our earlier study of baculovirus replication has resulted in the detection of small fraction of linear genomes in the sample of circular baculovirus DNA purified from *Antheraea pernyi* cells infected by *Malacosoma neustria* nuclear polyhedrosis virus (ManeNPV) [1]. "Linearization point" was localized at the particular site of ManeNPV genome. Cloning and sequencing of the "linearization point"-containing fragment followed by search for its homologs in GeneBank database has allowed us to identify the highly conserved non-protein coding element (CNE), 152-156 bp in length in the genomes of all representatives of genus *Alphabaculovirus* [2]. The consensus sequence resulting from the multiple alignment of 50 alphabaculovirus CNEs has demonstrated the complex structure of the element. The CNE consists of 7 blocks enriched by the

absolutely conserved nucleotides, some of these blocks are represented by the imperfect palindromes. The "linearization point" was localized within the CNE sequence. Positioning of the CNE in the genomes of sequenced viruses has revealed that the *Autographa californica* multiple nucleopolyhedrovirus (ACMNPV) CNE overlaps with previously described genomic elements of this virus, the transcription activator of ie2 gene and three genes of non-coding RNAs. The RNA-coding capacity of the CNE as well as its transcription-promoting activity are unlikely to be related to the genome linearization and, accordingly, it was suggested that some additional function may be attributed to the CNE. To test this suggestion the following experiments were performed.

The genome of AcMNPV was subjected to gene engineering manipulations to obtain the recombinant genomes: the CNE-knockout genome ($vAc^{CNE/Ko-EGFP}$), and the CNE-repaired genome where CNE was replaced 9 kb apart of its native location ($vAc^{CNE/Ko-REP-EGFP}$). The green fluorescent protein gene was inserted in the wild type and recombinant genomes to simplify the detection of infected cells by using fluorescent microscopy. All manipulations were performed using commercially available Bac-to-Bac system (Invitrogen) where the AcMNPV genome propagates in E.coli as a large plasmid. Transfection-infection assays were performed to check the ability of recombinant DNA to provide infection process. It was shown that the transfection of Sf9 cell monolayers by wild type DNA ($vAc^{EGFP}$ ) or $vAc^{CNE/Ko-REP-EGFP}$ led to the development of infection and production of infectious virus, whereas $vAc^{CNE/Ko-EGFP}$ failed to spread the infection from cell to cell. These data suggest that the CNE plays an essential role in virus pathogenesis and its essential function is not related to its RNA-coding capacity or cis-regulatory activity.

Bioinformatic approaches were applied to investigate the CNE structure and probable CNE clustering with other genomic elements in order to find some analogies with known functional elements of other viruses. The application of DNA fold software to structural analysis allowed to establish the regularity: despite of some variability in nucleotide content the vast majority of 50 CNEs demonstrate similar folding pattern, the long hairpin with low free energy that may reflect the potential of the CNE to structural transitions. The similar structures in respect of folding and size appear to be the characteristic feature of herpesvirus oris and poxvirus signals of DNA resolution from concatemeric replication intermediates (it is remarkable that the activities of both elements are associated with their structural transitions [3], [4]). The further comparative analysis of baculovirus genome contents led to the establishing of the following regularity: the CNEs of all 50 viruses were located between the promoters of bidirectional gene pair (it is remarkable that the CNE-associated genes

were not represented by homologs when distantly related alphabaculoviruses were compared). This finding corresponds to the model of virus oris, where adjacent transcription regulators play roles of auxiliary elements [5].

Multiple non-specific oris that function during the replication of virus DNA were identified in baculovirus genome earlier. The question remains open, however, whether the specific "initiating" ori is there. As the bioinformatic analysis data suggest that the CNE is a good candidate for ori, the transient replication assays were performed to check this suggestion. It was shown that AcMNPV CNE fails to support plasmid replication in Sf9 cells infected with AcMNPV. These data, however, could not exclude the possibility that CNE is a genuine "initiating" ori. The activity of such kind of ori can be predetermined by the highly condensed state of DNA within the capsid, and such state is out of reach in transient replication assays.

To further investigate the CNE role in viral pathogenesis an electron microscopy analysis of the cells transfected by $vAc^{CNE/Ko\text{-}EGFP}$ was performed. The resulting data demonstrated that $vAc^{CNE/Ko\text{-}EGFP}$ form nucleocapsids a great proportion of which was characterized by aberrantly long size. Long size of the nucleocapsids may be indicative of CNE involvement in DNA concatemer resolution, although the availability of nucleocapsids of normal size and morphology suggests that the blocking point of infectious virus formation is hardly related to the cleavage process. Further analysis revealed that the blocking point is an inability of both normal and aberrant nucleocapsids to leave the nucleus: all nucleocapsids were retained in the virogenic stroma occupying the central part of the nucleus and any of them were detected out of it. It was concluded that the CNE essential function is related to the nucleocapsid egress from the virogenic stroma region towards the nuclear periphery.


1. Kikhno I. Identification of a conserved non-protein-coding genomic element that plays an essential role in Alphabaculovirus pathogenesis. PLoS One – 2014. – 9, №4:e95322.
2. Строковская Л.И., Соломко А.П. Новый вид модификации юакуловирусных геномов в культуре клеток насекомых. Доповіді НАН України.- 1997.- №7. - С. 174-9.
3. Aslani A., Simonsson S., Elias P. Novel conformation of the Herpes Simplex Virus origin of DNA replication recognized by the origin binding hroteinhttp://www.jbc.org/content/275/8/5880.abstract?ijkey=0a64afc6fa6c22417d3ad85a306d8b2e5ed7da56&keytype2=tf_ipsecsha - fn-1 J Biol Chem – 2000-275, №8:5880-7.

4. Culyba M.J., Harrison J.E., Hwang Y., Bushman F.D. DNA cleavage by the A22R resolvase of vaccinia virus – 2006- 352,№ 2:466–76
5. DePamphilis. Eukaryotic DNA replication: anatomy of an origin. Annu Rev Biochem. 1993, 62::29-63.

# PHYLOGENETIC AND IN SILICO STUDY ON STRUCTURAL ELEMENTS OF REVERSE TRANSCRIPTION INITIATION IN RARE HIV-1 GROUPS AND SIV GROUPS

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The process of reverse transcription, a major obligatory step in the retroviral replication, is initiated from the 3′ end of tRNA primer annealed to the primer binding site (PBS) which is located in the 5′ untranslated region of the viral RNA (vRNA). This process is catalyzed by the virus-encoded reverse transcriptase (RT). All members of the primate lentivirus group, which includes a human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2) and simian immunodeficiency viruses (SIVs), employ the tRNAlys3 as a primer. The reverse transcription initiation implies the assembling the vRNA, tRNA primer, RT and nucleocapsid (NC) protein into the properly folded initiation complex. While a large volume of experimental data on the reverse transcription initiation is available for MAL and LAI/HXB2/NL4.3 strains of HIV-1 group M (major or main), this process is not extensively investigated in rare HIV-1 groups (N, O and P) and SIVs.

Among structural determinants of the HIV-1 reverse transcription initiation complex are the PBS/antiPBS interaction and two intramolecular duplexes of the vRNA – the U-duplex, bottom duplex of U5-top hairpin, and the D-duplex initially occluding the primer activation signal (PAS). The intermolecular interaction between the PAS motif of the vRNA and the antiPAS sequence of the tRNAlys3 (PAS/antiPAS interaction) was

demonstrated to be important for efficient initiation and a mechanism for negative and positive regulation of HIV-1 reverse transcription initiation through initial occlusion of the PAS motif by the D-duplex structure and its following exposure to anneal with the antiPAS sequence of the primer has been proposed [1]. It was shown that the availability of the PAS motif is substantially affected by the sequences downstream of the PBS, however structural rearrangements (in terms of base pairing) upon PAS exposure were not defined [2]. This interaction is conserved not only in HIV-1, HIV-2 and SIVs, but similar contacts (U5/TΨC-stem interactions) are possible in many other retroviruses, suggesting that the reverse transcription is initiated by a common mechanism in all retroviruses.

In this work, we conducted a phylogenetic comparison of the main structural elements of the reverse transcription initiation complex and defined structural rearrangements occurring upon PAS exposure in Mfold-calculated conformations of different fragments of U5-PBS region among three rare groups of HIV-1 (N, O and P) and three SIV groups infecting African great apes (SIVgor, SIVcpz*Ptt* and SIVcpz*Pts*). According to phylogenetic data on viral complete/partial genome or proteome sequences reported in literature, for example [3], HIV-1 groups fall within the HIV-1/SIVcpz/SIVgor radiation and each of HIV-1 groups has probably originated from a separate transfer of SIV from chimpanzee and gorillas into the humans.

For analysis, we selected all full-length genomic sequences containing a region corresponding to the U5/AUG domain of HIV-1 groups N, O, P, and SIVgor, SIVcpz*Ptt,* SIVcpz*Pts* which were available by the end of January, 2016 in the Los Alamos HIV sequence database (http://www.hiv.lanl.gov/). Totally, 64 genomic sequences have been examined, in particular, 8 of HIV-1 group N, 26 of HIV-1 group O, 2 of HIV-1 group P, 4 of SIVgor, 15 of SIVcpz*Ptt*, and 7 of SIVcpz*Pts*. Our alignment of the U5-PBS region by identifying the structural elements referred to the reverse transcription initiation demonstrated a high conservation both of D- and U-duplexes among all HIV-1 and SIV strains studied, while certain group/subspecies-specific deletions/insertions in the tract flanking the PAS motif covariate with those in the middle part of the pal-sequence flanking the antiPAS tract, which results in stable base-pairing at the bottom of the D-duplex (D-duplex extension) in rare HIV-1 groups N, O, P, SIVgor, SIVcpz*Ptt* of the 2$^{nd}$ and 3$^{rd}$ clusters and SIVcpz*Pts*. The D-duplex and its extension in these groups may be described as an irregular duplex interrupted by a bulge or an internal loop, but not a pal-hairpin as in HIV-1 group M and SIVcpz*Ptt* of the 1$^{st}$ cluster.

The RNA secondary structures were predicted using on-line program at the Mfold web server (http://mfold.rna.albany.edu) [4]. To

generate conformations with initial PAS occlusion ($PAS^{masked}$ conformations), we applied two starting constraints, the first prohibiting base-pairing in the PBS motif (to model PBS/antiPBS interaction with tRNAlys3) and also 3 nucleotides immediately upstream of the PBS (to model a free CAG junction between PBS- and U-duplexes) and the second forcing 8 base pairs in U-duplex (to model U-duplex structure), while the D-duplex (and its extension) folded on its own. To generate conformations with PAS exposure ($PAS^{exposed}$ conformations), we applied the third constraint prohibiting the PAS motif from base pairing as employed in [2]. Then, we selected most stable and compact $PAS^{exposed}$ conformations, classified them by a pattern of structural rearrangements accompanying PAS opening and compared their stabilities.

The four patterns of PAS exposure ($PAS^{GU/CGC\text{-}duplex}$, $PAS^{slide}$, $PAS^{open(d)}$ and $PAS^{open(AAAC)}$ conformations) were observed in Mfold-calculated $PAS^{masked}$ and $PAS^{exposed}$ conformations of HIV-1 and SIV groups under this study. In HIV-1 group M and SIVcpz*Ptt* of the 1st cluster, the PAS exposure occurs predominantly *via* $PAS^{GU/CGC\text{-}duplex}$ conformation. In HIV-1 group N and SIVcpz*Ptt* of the 2nd cluster, the decreasing order of stability is $PAS^{slide}$>$PAS^{open(d)}$. In HIV-1 group O and SIVgor of BQ cluster, the $PAS^{slide}$ and $PAS^{open(d)}$ conformations are very similar in structure and stability, $PAS^{slide}$≈$PAS^{open(d)}$. In HIV-1 group P and SIVgor of CP cluster, the most preferable $PAS^{open(AAAC)}$ conformation is highly specific for these strains and a less stable $PAS^{open(d)}$ conformation is also adopted, $PAS^{open(AAAC)}$>$PAS^{open(d)}$. In most strains of SIVcpz*Ptt* of the 3rd cluster, the $PAS^{open(d)}$ conformation is the most preferable and the only one, while a few strains of this group can also adopt the $PAS^{slide}$ conformation, $PAS^{open(d)}$>$PAS^{slide}$. In SIVcpz*Pts*, the $PAS^{open(d)}$ conformation is the most preferable and the $PAS^{GC/CGC\text{-}duplex}$ conformation is less preferable, $PAS^{open(d)}$>$PAS^{GU/CGC\text{-}duplex}$.

It appeared that our phylogenetic data and Mfold-predicted data on four schemes of PAS exposure well correlate with the phylogenetic relationships between HIV-1, SIVcpz and SIVgor constructed using *gag* and *pol* sequences which are reported in literature. This agreement is believed to reflect the interrelation between peculiarities, on the one hand, of *gag* and *pol* genes encoding among others structural NC protein and RT enzyme which are both crucial for the reverse transcription initiation process and, on the other hand, structural features of the observed $PAS^{exposed}$ conformations of the U5-PBS region which are also relevant to this process.

1. Beerens, N., Groot, F., & Berkhout, B. (2001). Initiation of HIV-1 reverse transcription is regulated by a primer activation signal. *Journal of Biological Chemistry*, *276*, 31247–31256.

2. Ooms, M., Cupac, D., Abbink, T.E.M., Huthoff, H., & Berkhout, B. (2007). The availability of the primer activation signal (PAS) affects the efficiency of HIV-1 reverse transcription initiation. *Nucleic Acids Research*, *35*, 1649–1659.
3. D'arc, M., Ayouba, A., Esteban, A., Learn, G.H., Boué, V. et al. (2015). Origin of the HIV-1 group O epidemic in western lowland gorillas. *Proc Natl Acad Sci U S A, 112,* E1343–E1352.
4. Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Research, 31*, 3406–3415.

# ISOLATION OF BACTERIOPHAGES CAPABLE TO LYSE *SERRATIA MARCESCENS* AND EVALUATION OF THEIR ACTIVITY ON ONION AND GERANIUM

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Plant pathogenic bacteria cause many serious plant diseases throughout the world stimulating intensive research of their ecology, pathology and epidemiology. Bacterial rot (*Erwinia carotovora*) and vascular bacteriosis (*Xanthomonas campestris*) cause epiphytoties and lead to large harvest destruction, resulting in requirement of the import of cabbages, tomatoes and pepper [1]. Bacteria of genuses Salmonella, Serratia, Enterobacter and Enterococcus, more frequently happen in the cases of nocosomial infections, e.g. food poisonings and sepsises [2-5]. The last researches, however, prove that these human pathogenic bacterial species also have ability to colonize a wide spectrum of plants and cause the disease development. For the instance *Serratia marcescens*, common soil bacteria, was described as causative agent of soft rot of onion [6]. However, it should be noted that the majority of these experiments were performed under laboratory conditions, while the development of crop diseases, caused by these pathogens in the environment, is still poorly understood. Our purpose was to search

bacteriophages, specific to *S. marcescens* and to verify their activity against this bacterium on the plants of onion and pelargonium.

In order to isolate bacteriophages the samples of plant displaying the symptoms of bacteriosis were used. To amplify putative bacteriophages in samples enrichment method was applied. The samples were plated on a bacterial lawn by agar overlay method. As result, we observed the formation of plagues with different morphology. Two bacteriophage isolates differed for the morphology of their negative colonies were selected for subsequent research. Phages that were in sample №5 resulted in formation of large and small negative colonies. Large colonies have round, smooth shape, d=5-6 mm, while small colonies have irregular shape, d=1-2 mm. We choose phages with small colonies because of their capability to accumulate in high titers compare to phages with large colonies. These phages were named as "S" in the experiment. Separate phage plagues were picked and transferred to sterile saline. Isolated bacteriophages were purified by serial propagation of single plaques. According to the results of spot-tests the titer of viruses after three passages was $10^{-7}$. High titer lysates were routinely prepared from confluent lysis plates. Then we studied the morphological features of the selected bacteriophages using electron microscopy. The isolate of S belongs to family of *Myoviridae* of order *Caudovirales* (morphotype A2).

The next stage was to verify if the selected bacteriophage isolate S was active against *S. marcescens* in test-system of onion scales. For this purpose scales were obtained from sterilized onion bulbs and incubated in plates on paper filter discs. The onion scales were scratched with sterile scalpel. In control variants, bacterial solution or physiological solution was inflicted into places of incisions (bacterial and intact control, respectively). In experimental variants, the scales were treated with nightly culture of bacterium and phage. All plates were placed in the incubator at 25$^{o}$C. In two days after inoculation we observed the development of soft rot on onion scales threated with bacterium and the absence of bacterial growth in the plate where phage was added. Thus, our phage isolate was effective against *S. marcescens* in selected test system.

In order to research the potential of isolated bacteriophages as therapeutic agents another model system was used. The ability of bacteriophages to suppress the development of bacteriosis was investigated on geranium plants. For this purpose geranium leaves were infected with bacteria in two ways: injection into veins with subsequent incubation in water or adding bacterial suspension to water. We utilized different ways for the treatment of plant leaves with bacteriophage preparations. In the first group the leaves was inoculated in a vein with the suspension of bacterium and phage, in the second group plant leaves were inoculated with a

bacterium into a vein and incubated in water supplemented with phages. As a result, bacteria cased redness of leaves in five days after inoculation. The symptoms were the same in both variants of bacterial infection. Turning red was observed along the veins while the whole leaf plate remained green. In the site of bacteriophage inoculation we observed clean area. This suggested that bacteriophage repressed bacterial growth. However according to data obtained isolated phage was limited in the ability to spread through the plant tissue.

Consequently, the probed bacteriophages repressed the development of phytobacteriosis caused by *S. marcescens* on onion scales and geranium leaves. The use of bacteriophages to combat bacterial infections may help to solve the current problem of antibiotic resistance. For successful application of bacteriophages fundamental issues arising from the ecological dynamic of host, bacterium and phage should be investigated in detail.


1. Фітопатогенні бактерії. Бактеріальні хвороби рослин: монографія. Т. 1 / Р.І. Гвоздяк, [et al]. - НАН України, Ін-т мікробіології і вірусології ім. Д.З. Заболотного. – К., 2011. – 444 с.;
2. Prasannath K. Pathogenicity and Virulence Factors of Phytobacteria // Scholars Academic Journal of Biosciences. – 2013. – Vol. 1, Is. 1. – P. 24-33.;
3. Heather L. T. Plants as a Habitat for Beneficial and/or Human Pathogenic Bacteria / Heather L. T., Triplett E. W.;
4. Kirzinger M.W.B. Insights into Cross-Kingdom Plant Pathogenic Bacteria / Kirzinger M.W.B., Nadarasah G., Stavrinides J. // Genes. – 2011. – Vol. 2. – P. 980-997;
5. A mobile genetic element in Serratia marcescens, a causative agent of onion disease / Ovcharenko L.P. [et al]. // Biopolymers and Cell. – 2010. – Vol. 26, N 4. – P. 279-285 ;
6. Janda J.M., Abbott S.L. The Enterobacteria, 2nd ed. – Washington D.C.: ASM press, 2006. – 411 p.

# MOLECULAR METHOD OF IDENTIFICATION OF FAMILY PHYCODNAVIRIDAE VIRUSES

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Viruses which are related to family Phycodnaviridae have a great potential in theoretical surveys (many new unknown genes, some unique life cycles et.) and in practical method of using (recovery from toxic «blossoms» of some algae). Nevertheless they are not enough have studied yet. But for full and complex researching of this family there are need molecular based technique of identification.

It is well known, that main molecular method for identification of biological agents is PCR. Other molecular methods are based on this method. In particular, for identification of questionable group of viruses – LNCDV, scientists are used primers, which are complementary to gene of DNA-depend-DNA-polymerase.

In this case, in the present day, there are some developed technique for identification of Phycodnaviridae. For example, for identification of *Prasinoviruses* genus, authors [1] developed two sets of primers: one for DNA-depend-DNA-polymerase and another one for MCP (major capsid protein). Of course, for identification they are used pyrosequencing which in turn to NGS (Next generation sequencing), a certain method of 454 Life Sciences. Also, using this method with primers to MCP and DNA-depend-DNA-polymerase, researchers found viruses from *Phycodnaviridae* family in South sea of Korea [4], and found *Prasinovirus* , *Prymnesiovirus* та *Cocolithovirus* too. Besides of this, good marker for identification of *Phycidnaviridae* is presence of intein in their DNA-depend-DNA-polymerase gene [2]. Of course, there is a developed primer for intein too.

So, we can see some success of identification this family Phycodnaviridae, using a classical method - PCR. But in the present time there are emerge some new molecular method of identification. Thanks to this method, we can do it more efficient, faster and cheaper. This methods are connected by name «Isothermal amplification» [3]. There are some of them: LAMP, NASBA, RPA, HDA and other. So, these primers were developed for most members of family Phycodnaviridae, it also means that we can use it for this new technique because basic principle is the same as in PCR.

In the consequence, application of this new methods called «Isothermal amplification» for identification Phycodnaviridae help us to do this faster, cheaper, efficient and more accuracy.

1. Clerissi C. et al / Unveiling of the diversity of Prasinoviruses (Phycodnaviridae) in marine samples by using high-throughput sequencing analyses of PCR-amplified DNA polymerase and major capsid protein genes. // Appl Environ Microbiol. – 2014; 80(10):3150-60
2.Culley al. et al / Detection of inteins among diverse DNA polymerase genes of uncultivated members of the Phycodnaviridae.
// ISME - J 2009;3(4): 409-18;
3. Gill P Ghaemi A. / Nucleic acid isothermal amplification technologies: a review. // Nucleosides Nucleotides Nucleic Acids. – 2008; 27(3):224-43.

## FIRST EVIDENCE OF THE WIDE SPREAD OF *TURNIP MOSAIC VIRUS* IN UKRAINE: THE MISSING LINK OF EVOLUTION?

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*Turnip mosaic virus* (TuMV) is a member of *Potyvirus* genus belonging to the largest *Potyviridae* family of plant viruses. For domesticated *Brassica* plants, TuMV is considered one of the most damaging and economically important viruses [1]. TuMV probably occurs worldwide and has been found in both temperate and subtropical regions of Africa, Asia, Europe, Oceania and North and South America [2, 3]. Despite Ukraine's geographical location and wide cultivation of different *Brassica*

crops for centuries, TuMV has never been registered on field crops in this country. The only published record of TuMV occurrence in Ukraine describes a single virus-infected sample of wild orchid *Orchis purpurea* from the Crimea peninsula [4]. A phylogeographic analysis showed that TuMV supposedly has originated in western Eurasia and/or North Africa, and probably evolved from a virus of monocotyledonous plants, presumably orchids [5]. In this context, Ukraine is one of the possible candidates for 'centre of emergence' of TuMV.

A total of 54 plant samples with TuMV-like mosaic and mottling symptoms were collected in different districts of the city of Kyiv and Kyiv region. TuMV has been detected in 27 samples of plants (overall 50% incidence rate in symptomatic hosts) including *B. oleracea* var. capitata, *R. sativus*, *Raphanus* sp., *S. alba*, *B. juncea*, *C. sativa*, *Brassica* sp., and *Bunias orientalis*. On cabbage plants, TuMV typically induced vein banding and leaf deformation, whereas systemic mosaics and mottling were common for naturally infected radish and mustard plants.

Detailed study of the host range of UKR9 isolate of TuMV indicated that it infected systemically various *Brassica* (cabbage, Chinese cabbage, kohlrabi, mustard, rape, turnip, etc.) and *Raphanus* (Chinese and Japanese radish) plants. Therefore, this isolate was regarded as belonging to BR pathotype.

The genomic nucleotide sequence of UKR9 isolate was determined. The sequence of the isolate was 9833 nt long. The polyprotein sequence was assessed for evidence of recombination. A 'clear' interlineage recombinant nature of UKR9 isolate (*P* values smaller than $1x10^{-6}$) was confirmed using the RDP ($3.68 \times 10^{-21}$), BOOTSCAN ($2.83 \times 10^{-21}$), MAXCHI ($3.12 \times 10^{-11}$), CHIMAERA ($2.14 \times 10^{-13}$) and SISCAN ($6.42 \times 10^{-14}$) algorithms in RDP4 software, when GENECONV algorithm indicated tentative recombinant nature of UKR9 (*P* value of $3.76 \times 10^{-2}$). Therefore, UKR9 was at least a single recombinant and an interlineage recombinant of World-B (Rn98) x Asian-BR (TUR9) parents (detected as minor and major parents, respectively).

Phylogenetic analysis confirmed that Ukrainian isolate UKR9 was genetically distinct from both parental isolates found in Italy (Rn98, minor parent) and Turkey (TUR9, major parent), and a novel isolate.

In summary, the survey indicated high occurrence of TuMV in urban and agricultural territories where infection incidence rate reached 50%. Wide range of infected plant species in surveyed areas suggests a just discovered long-term coexistence of the virus and the hosts in Ukraine which is one of the logistic hubs between Europe and Asia, and possibly is another hotbed for TuMV evolution. These results may be supportive of our hypothesis that TuMV spread may eventually follow the Silk Road.

However, to our knowledge, the present study shows for the first time the wide distribution and evolutionary relationships of TuMV from plants collected in Ukraine.


1. Walsh J.A., Jenner C. E. Turnip mosaic virus and the quest for durable resistance // Molecular Plant Pathology. -2002. -3. –P.289–300.
2. Provvidenti R. Turnip mosaic potyvirus. In: Brunt A.A., Crabtree K., Dallwitz M.J., Gibbs A.J., Watson L. (eds.) Viruses of Plants. - Wallingford, UK: CABI, 1996, p.1340–3.
3. Ohshima K., Yamaguchi Y., Hirota R., Hamamoto T., Tomimura K., Tan Zh., Sano T., Azuhata F., Walsh J.A., Fletcher J., Chen J., Gera A., Gibbs A. Molecular evolution of Turnip mosaic virus: evidence of host adaptation, genetic recombination and geographical spread // Journal of General Virology. -2002. -83. –P.1511–1521.
4. Korotieieva H.V., Polishchuk V.P. Viruses of orchids of the natural flora in Ukraine // Mikrobiol. Z. -2004. -66(2). –P.74-80.
5. Gibbs A.J., Ohshima K. Potyviruses in the digital age // Annu. Rev. Phytopathol. -2010. -48. –P.205–223.


# THE DRUG MIXTURE OF BACTERIOPHAGES TO PREVENT AND CONTROL BACTERIAL DISEASES OF POTATO


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The prevention and control of bacterial agricultural plant diseases is carried out by using a mixture of bacteriophages specified to bacteria strains. Infected seeds, diseased plants or plant material, the soil in which plants are grown are treated by bacteriophages, also adding to the irrigation water, as well as to the soil, soil additives. The main advantage is to create drugs and prevention from bacterial diseases of plants, alternative methods of chemical germicides which pollute the environment. Using chemical microbicides helps to control the development of these pathogens but in some cases the efficiency of their use is quite low, these drugs are often

unsafe for plants and the environment. Lytic bacteriophages, bacterial viruses that selectively kill and lyse phytopathogenic bacteria are an effective alternative for controlling plant diseases.

Objective: to investigate the properties of bacteriophage isolates found in potato with features of bacteriosis in order to test the prospects for their using in antibiotic therapy.

To start with, amount of bacteriophages was described as a promising drug to cure plant bacterial diseases.

Four bacteriophage isolates were isolated from samples that have symptoms of bacterial disease (and were previously infected with bacteria of *Pseudomona* genus). Phages were isolated through direct seeding. Titers were defied in colony-forming units in 1ml (PFU\ml) using double agar layer method (by Gracia). Clean bacteriophage lines were received by passaging them six times. In order to determine the host range of isolates a research of lytic activity phage range was conducted of 15 strains of pathogen bacteria. The research process revealed that such isolates are polyvalent. Polyvalent phages are able to affect not only strains belonging to one bacteria species but also different species, genera, and groups of bacteria. Polyvalence has fundamental value in virology, since some especially dangerous infections persist in the environment due to the expansion of the respective viruses' hosts, so as the significance of this phenomenon for control of the number of owners [1]. This ability can become a key feature in a struggle with dangerous bacterial pathogens in different spheres of a human life, including medicine, farming industry, and food industry.

Morphology of viral particles was studied with a help of electron microscope (JEOL JEM - 1400). Electronogram analysis showed that studied phages were slightly different in both virion structure and size. Among them, a group of phages was identified as a *Podoviridae* genus and a *Caudovirales* order (icosahedral head with a short shoot and a small size: 43±1 nm head diameter and 1±0,5 nm tail length). More than 70 species, currently known, belong to 20 genera [2].

Phages proteins fractionation was conducted by method of disc electrophoresis (by Lemmli) in polyacrylamide gels (PAGE) [3]. Major capsid proteins of investigated phages are within 35-45 kDa in regard to the isolate. These molecular weights close with a molecular weight of Pseudomonas aeruginosa phage KPP21 isolated in Japan which according to morphological and genetic analysis belong to the family Podoviridae N4 – like phages [4]. Thus, a comparative analysis of the protein composition of 4 investigated phages which is represented by a set of minor and major polypeptides had shown some disagreement on this basis. Indicators of molecular weight were between polypeptide 14 – 116 kDa and the number

ranged from 11 to 15. Moreover, 9 structural proteins are typical for *Podoviridae* family. More variants of proteins could possibly be result from an additional frameshift event or from posttranslational modification [5].

As a result, a drug of bacteriophage which is able to lyse bacteria of the genus *Pseudomonas* was obtained. Isolate concentration of bacteriophages is polyvalent: it reduces the prevalence of plant bacterial diseases by 50-90% and improves plant productivity.

1. Davison A. J., Benko M., Harrach B. Genetic content and evolution of adenoviruses // J. Gen. Virol. – 2003. – 84. – P. 2895–2908.
2. ICTV. "Virus Taxonomy: 2015 Release". Retrieved 1 July 2015.
3. Methods of Protein and Nucleic Acid Research Volume 1: Electrophoresis Isoelectric Focusing Ultracentrifugation. Osterman, L.A. - 1984
4. Shigehisa R. Uchiyama J., Kato S. Characterization of Pseudomonas aeruginosa phage KPP21 belonging to family Podoviridae genus N4-like viruses isolated in Japan // Microbiology and Immunology. - 2016. - Vol. 60 - p. 64-67.

5. Hans Petter Kleppen, Helge Holo,Sang-Rok Jeon Novel *Podoviridae* Family Bacteriophage Infecting *Weissella cibaria* Isolated from Kimchi // Appl Environ Microbiol. - 2012. - № 78 (20) - p. 7299 – 7308.

## DIS, SD AND Psi HAIRPINS IN HIV-1 AND SIV GENOMIC RNAs

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The genome of human immunodeficiency virus of type 1 (HIV-1), the causative agent of the acquired immunodeficiency syndrome (AIDS), is highly heterogeneous. HIV-1 strains are classified into four groups: M, N, O and P. HIV-1 M is responsible for the global HIV/AIDS pandemic and subdivided into 9 subtypes (A-D, F-H, J and K), four subsubtypes (A1, A2,

A4, F1 and F2), 79 circulating recombinant forms (CRFs) and many unique RFs (URFs). The genome dimerization controls recombination and plays an important role in promoting the HIV-1 genetic diversity. Genomic RNAs dimerize via the interaction between DIS (dimerization initiation site) hairpins [1]. The DIS hairpin located in the 5′ untranslated region of HIV-1 genome contains a palindrome and flanking purines in its apical loop. Intermolecular Watson-Crick interaction between two palindromes leads to the formation of the "kissing loop" dimer and flanking purines are essential for this process.

In this work, we conducted a large scale comparative study of the secondary structures of the DIS hairpin and adjacent SD (splice donor) and Psi (packaging signal) hairpins in the genomes of human and simian immunodeficiency viruses. The secondary structures of the region encompassing these hairpins have been predicted by the UNAFold program. The genomic sequences of this region have been extracted from the NCBI database. About 4900 HIV-1 M genomes from ~1800 different patients and 28 SIV genomes from 22 different apes have been analysed. Among HIV-1 M genomes, we found 266, 150 and ~200 different variants of the DIS, SD and Psi hairpins, respectively, that occur with various frequencies. The DIS sequence length is commonly 35 nucleotides (nts). The common 19-nt SD hairpin is extended by an additional bottom base pair in ~10% genomes. The Psi sequence length is commonly 27-29 nts. In ~15% HIV-1 M genomes (mainly of C subtype), the Psi hairpin is truncated up to 14 nts.

Despite sequence variation among HIV-1 M isolates, the secondary structures of the DIS, SD and Psi hairpins are well conserved. We have classified all HIV-1 M genomes into five groups by the apical loop of parental DISes ($DIS_{Lai}$, $DIS_{Trans}$, $DIS_F$, $DIS_C$ and $DIS_{Mal}$). These apical loops are AAGCGCGCA, AAGUGCGCA, AAGUGCACA, (A)AGUGCAC(U) and AGGUGCACA, respectively. The palindromes are underlined and the nucleotides involved into the upper base pair are in brackets. $DIS_{Trans}$ may evolve from $DIS_{Lai}$ *via* a single base change in the apical loop, as well as $DIS_F$ – from $DIS_{Trans}$ and $DIS_C$ – from $DIS_F$. $DIS_{Mal}$ may evolve *via* a single base change from $DIS_F$ variant with G23A mutation in the hairpin stem. HIV-1 M isolates with $DIS_{Lai}$, $DIS_{Mal}$, $DIS_C$ and their variants are widely spread. About 90% genomes with $DIS_{Lai}$ belong to B and D subtypes (which can be considered as subsubtypes of the same subtype), the rest being B/D-containing recombinants. HIV-1 genomes with $DIS_{Trans}$ belong to B subtype and B/D/F-containing recombinants. About 90% of genomes with $DIS_C$ belong to C subtype. HIV-1 genomes with $DIS_{Mal}$ belong to A/E/G-containing subtypes (A, G, CRF01_AE, CRF02_AG and others). $DIS_F$ can be considered as a source of all DIS variants. It can evolve to all other parental DISes *via* single or double mutations. HIV-1 M genomes with $DIS_F$

belong to F subtype (~20%), C subtype (~30%), CRF02_AG (~10%), G, H, J subtypes and C/F/G/H-containing recombinants.

Base changes at certain positions of the parental DISes occur with different frequencies. For example, G23A base change leading to U:G→U:A base pair substitution is found in 42% HIV-1 M genomes with $DIS_{Lai}$, 33% – with $DIS_F$ and only 2% – with $DIS_C$. Certain base changes in the parental DISes are supposed to differently affect their stability, exposure of palindrome, nucleocapsid protein (NC) affinity and compatibility between stem conformation and apical loop conformation. Base changes at certain positions of SD and Psi hairpins also occur with various frequencies in HIV-1 M genomes with different parental DISes.

The overall architecture of the DIS and Psi hairpins in HIV-1 nonpandemic group N genomes is similar to that in HIV-1 M. The DIS hairpin in HIV-1 N has the apical loop GUGCACA, the Psi hairpin is predominantly in the truncated form. However, the SD hairpin containing the major splice donor site coexists in several forms and significantly differs from those in HIV-1 M. The Psi hairpin in HIV-1 M and HIV-1 N is located immediately upstream of GAG start codon. HIV-1 O and HIV-1 P genomes contain an additional hairpin which is inserted downstream of the truncated Psi hairpin. A function of this hairpin is not determined yet. We named it as $SL^{INS}$. The DIS and truncated Psi hairpins in HIV-1 O/P genomes are similar by the overall architecture to those in HIV-1 M and HIV-1 N genomes, while the SD hairpin is very similar in HIV-1 O and HIV-1 P, but different from those in HIV-1 M and HIV-1 N.

SIVs infecting chimpanzee *Pan troglodytes troglodytes* (SIVcpz*Ptt*) and gorilla (SIVgor) are considered as the simian precursors of HIV-1 [2]. SIVgor is suggested to be the ancestor of both HIV-1 O and HIV-1 P, and gorilla may also be an intermediate host between chimpanzee and human. We found that all SIVcpzPtt genomes studied can be divided into 3 main groups according to the structure of the region encompassing DIS, SD, Psi and $SL^{INS}$ hairpins. The structure of this region in SIVcpz*Ptt* genomes of the 1st group (5 genomes) is very similar to that in HIV-1 M genomes, contains $DIS_F$, $DIS_{Mal}$ or $DIS_{Trans}$ variant with several base changes, common (19 nts) or an extended SD hairpin (21 nts), a long Psi hairpin (29-31 nts) and lacks $SL^{INS}$. The DIS and SD hairpins in the SIVcpz*Ptt* of the 2nd group (2 genomes) are similar to those in HIV-1 N, while the long Psi hairpin contains a subspecies-specific insert AGU/CA at the bottom part. All SIVcpz*Ptt* genomes of the 3rd group contain the $SL^{INS}$ hairpin and can be divided into two subgroups. The DIS ($DIS_F$ and $DIS_{Mal}$ variants) and SD hairpin (commonly with C19A base change) of the 1st subgroup (8 genomes) resemble those in SIVcpz*Ptt* of the 1st group, while the Psi hairpin is truncated due to the $SL^{INS}$ hairpin presence. As distinct from the

1st subgroup, the SD hairpin of the 2nd subgroup (4 genomes) has a specific structure.

SIVgor isolates are divided into 3 clusters (CP, BP and BQ). We found that the secondary structure of the region under study in all three clusters and HIV-1 O/P is similar. In particular, this region structure in SIVgor-BQ is almost identical to that in HIV-1 O isolate ANT70 and it in HIV-1 P is very similar to that in SIVgor-BP. Our distribution of SIV genomes into groups/subgroups according to the structure of the region encompassing DIS, SD and Psi ($SL^{INS}$) hairpins coincides with the phylogenetic relations across HIV-1 and SIV strains based on *gag*/GAG sequence, for example [2].

Thus, among all HIV-1 and SIV genomes studied, we demonstrated a high conservation of the DIS and truncated Psi hairpins, which may indicate that both the sequence and secondary structure of these hairpins are important for HIV/SIV replication. As to the SD hairpin, the sequence of the major splice donor site is more important for virus replication than the definite structure of this hairpin. The fact that the DIS hairpin in SIV genomes commonly possesses the GUGCAC palindrome supports our proposal that the parental $DIS_F$ can be considered as a source of all DIS variants. Although the Psi hairpin in HIV-1 O/P, SIVgor and SIVcpz*Ptt* genomes of the 3rd group is truncated due to the $SL^{INS}$ hairpin, an oligo(U) and G/A-rich stretches, which are involved into the formation of the bottom part of the long Psi hairpin, are preserved, that may evidence a functional importance of these stretches. The present comparative study on the DIS, SD and Psi hairpins which are important for HIV-1/SIV replication can facilitate the detection of HIV-1 groups and subtypes which may be overlooked at present or appear in future.

1. Moore MD, Hu WS. HIV-1 RNA dimerization: It takes two to tango. AIDS Rev. 2009; **11** (2): 91- 102.
2. D'arc M, Ayouba A, Esteban A, Learn GH, Boué V et al. Origin of the HIV-1 group O epidemic
in western lowland gorillas. Proc Natl Acad Sci U S A. 2015; 112(11):E1343-52.

# MORPHOLOGICAL HETEROGENEITY OF TEMPERATE ERWINIOPHAGE 59

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Investigating temperate erwiniophage 59 [1] we revealed the phenomenon of morphological populational heterogeneity. When propagated on various sensitive strains, the phage has produced two equimolar subpopulations with different buoyant density. Thus, the aim of this research was to characterize the populational heterogeneity of erwiniophage 59 obtained on different isogenic strains of the host microorganism.

Erwiniophage 59 was obtained by propagation on its traditional host *Erwinia "horticola"*(*Eho*) 450, as well as on its three isogenic strains and a related bacterium *Eho* 60. Physical and chemical properties of the phage particles were studied using centrifugation in CsCl-gradients, electrophoresis in agarose gels, electron microscopy, restriction analysis of DNA and SDS-PAGE of the virion polypeptides.

In the earlier studies [2] it was shown that phytopatogenic bacteria are characterized by the presence of structural heterogeneity of virions within their populations. Similar results were obtained in current investigation for temperate erwiniophage 59 in the case of reproduction in cells of the auxotrophic mutant *Eho* 450 His3. The pool of phage particles produced two equimolar bands in CsCl gradient with density of 1.45g/cm$^3$ and 1.47g/cm$^3$.

For simplicity of notation, we assume to designate the virus-containing material of bands with the lower and higher density as subpopulation I (SPI) and subpopulation II (SPII) respectively.

The electrophoretic separation of the summary sample and the analysis of SPI and SPII contents showed that the general pool of phage particles is characterized by two groups of virions. The electrophoretic mobility of DNA molecules extracted with SDS and pronase B was the same in all cases. Identical restriction patterns were obtained using *Sal*I restriction enzyme for both subpopulations. DNA fragments have the same size and relative content.

The study of plaque morphology inferred that SPI and SPII produce morphologically identical plaques on the lawn of sensitive bacteria.

Phage plaques were characterized by turbid centers, clear halos and uniform sizes. The fact of different lysogenization frequency of *Eho* 450 His3 by SPI and SPII phage has been established. This index differs by 2 orders and equals 3.3 and 1.7 % for SPI and SPII phage particles respectively.

Lysogenic strains obtained using *Eho* 450 His3, SPI and SPII phages were shown to be resistant to homoimmune superinfection and cross-infection. Furthermore, at least two stable lysogenic types of the host-microorganism were detected. The first lysogen formed small zones of bacterial lysis on the lawn of sensitive culture while the second lysogenic colony was characterized by big lysis zone of the indicator strain. Subpopulation I and II produced both lysogenic types, but the percentage of the second type lysogen was several orders of magnitude smaller. The isolated lysogens differed by the frequency of bacteriophage spontaneous induction. The spontaneous induction frequency of the first type lysogen after 20 h of incubation reached $2Ч10^{-2}$. For the second type lysogen this index differed by two-orders of magnitude ($3Ч10^{-4}$).

The average values of the maximum capsid diameter and the tail length were established for SPI and SPII virions by means of electron microscopy. The maximum diameter of the phage capsids equaled 51.16 ± 0.23 nm for SPI and 55.36 ± 0.57 nm for SPII capsids, which is 8.2 % larger than the one for SPI capsids.

Next, the host influence on the adaptation process and the phage morphogenetic development were investigated. The adaptation was performed by means of 4–5 serial clonings through a single plaque on an appropriate strain. Gradient profiles of the recloned phages indicate that all the studied strains produce heterogeneous phage pools characterized by quantitative redistribution of the viral material between SPI and SPII depending on the strain and the number of reclonings. Thus, strains *Eho* 450, $450(P1)^-$ and 450(49) produce bands of equal intensity in the CsCl-gradient. The lower band is dominant in 59/450 His3 and 59/450(49) preparations while for 59/60 it is the upper one. It is worthy to note that the virus sample recloned and obtained using strain *Eho* 450 have a slightly visible top band. Such redistribution of virion classes within the population may be an evidence of the tendency to homogeneity with subsequent passages when grown on an adequate host.

Based on the electrophoretic data it was determined that SPI and SPII phage particles are characterized by identical polypeptide profiles. The phage virions include at least 8 structural polypeptides with molecular weights ranging from 11 to 126 kDa. Three of them (p4, p5, p7) are the major ones. The difference in percentage correlation between p4 and p7 has been established for SPI and SPII phage particles.

The influence of the indicated structural changes on the packaging and permutation of DNA was determined by using restriction analysis. The

*Sma*I endonuclease fragments of SPI and SPII DNA had the same electrophoretic mobility. The *Sma*I digests of the phage 59 DNA contains six basic fragments, the set of heterogeneous fragments ("a") and one submolar fragment ("b").

In summary, it was shown that the capsid diameter of the particles with higher density (SPII) equals 55.36 nm, which is 8.2 % larger than the one for SPI capsids. Both types of particles do not differ by DNA size and have identical restriction patterns. Based on the *Sma*I-restriction analysis it may be concluded that the DNA packaging remains unchangeable and is carried out according to the head-full packaging mechanism. The difference in percentage correlation between p4 and p7 probably is related to the distinction in capsid structures; thus, SPI and SPII phage particles have different protein-DNA correlation. This may possibly explain the electrophoretic mobility variation of the virions within a particular population. Curiously, the subpopulations I and II have different values of lysogenization and spontaneous induction frequencies

The reasons for the occurrence of an additional phage particle set within the phage 59 population remain unclear and require further investigation. It may be supposed that the morphological heterogeneity may be caused by changes in the phage genes as well as by the conditional changes of the infection process. One should not also exclude that the new subpopulation appears as a reaction of the pseudolysogenic system *E.horticola* to the infection by a homologous phage 59.

1. Kishko Ya.G., Ruban V.I., Tovkach F.I., Murashchyk I.G., Danileychenko V.V. Structure of Erwinia carotovora temperature bacteriophage 59 and its DNA // J Virol. – 1983. – 46, No 3. – P. 1018–1021.4.
2. Korol N.A., Romanyuk L.V., Ostapchuk A.N., Ivanytsa T.V., Kushkina A.I., Tovkach F.I. Pecularities of morphogenetical development of virulent mutants of erwiniophage ZF40 // Mikrobiol Z. – 2011. – 73, No 2. – P. 58–64.

# SESSION
# “VETERINARY VIROLOGY”

# ANALYZING OF PORCINE EPIDEMIC DIARRHEA VIRUS DISTRIBUTION IN UKRAINIAN FARMS SINCE 2014-2016

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Porcine epidemic diarrhea virus (PEDV), an *Alphacoronavirus* in the family *Coronaviridae*, causes acute diarrhea, vomiting, dehydration, and high mortality rates in neonatal piglets [1]. PEDV can infect pigs of any age, from neonates to sows or boars; however, the severity of PED in pigs differs according to age. PEDV was first described in Europe in the 1970s and since then has spread over many Asian and American countries. In April 2013 major outbreaks of PEDV have been reported in the United States, Canada, and Mexico. From this period economic loses in US resulting in 10% of pig production [1, 2].

In Ukraine PEDV was detected in 2014 at first. Since that time, virus was found in different regions with doubled infection. Importantly, that pathogenicity of PEDV in different continents, countries, sometimes regions and period are differing according to virus strains. That's why the aim of the study was to analyze distribution of Porcine epidemic diarrhea virus in Ukraine farms from 2014 to 2016.

In present study we have analyzed 707 samples from pigs with diarrhea disorders. All samples were collected from swine of different age group and regions of Ukraine since 2014 to 2016. The detection and taste sampling were performed in Veterinary Diagnostic Center of Kyiv.

During 2014 we have examined 210 samples from Dnipropetrovsk, Zhytomyr, Kyiv, Cherkasy, Poltava, Sumy and Zakarpatska regions. As a results PEDV positive samples were determined in 41 (20%) from all analyzed material. Therefore in 2015 the situation with detection and distribution was changed to the side of virus spreading. In general with the help of molecular detection there were analyzed 473 samples from Dnipropetrovsk, Vinnytsia, Zhytomyr, Zakarpattya, Ternopil, Chernivtsi, Kyiv, Cherkasy, Chernihiv and Poltava regions. The biggest amount of PEDV positive samples were detected in Poltava 47 (10%) and Kyiv – 38 (5%) regions. As the results in 2015 we noticed virus destruction to the west of Ukraine and show it emergence. During our researching from January to May 2016, the presence of PED virus were observed in 8 samples out of 23 tested animals from 5 regions of Ukraine (35%). Moreover, the positive

samples were collected from three regions of Ukraine, namely Kyiv, Cherkasy and Odessa. In the Poltava region, unlike previous years, all tested samples were negative in PEDV.

As a results for 3 years of studying we have confirmed detection of PED positive farms and show distribution of Alfacoronavirus in Ukraine. At first PEDV was detected in the center of Ukraine in Poltava region. From our investigation we have seen that virus spread to another regions (Cherkassy, Kyiv and Odessa). But in date of 2016 we marked extremely positive trend in reducing of PEDV spreading in Ukrainian farms.

1. Daesub Song, Hyoungjoon Moon, Bokyu Kang. Porcine epidemic diarrhea: a review of current epidemiology and available vaccines // Clinical and Experimental Vaccine Research., - 2015. – Vol. 4 – P. 166-176.

2. Rui-Qin Sun. Outbreak of Porcine Epidemic Diarrhea in Suckling Piglets, China // Emerging Infectious Diseases . – 2012. - Vol. 18. – pp. 1-10.

# DETECTION AND DIFERENTIATION OF NEWCASTLE DISEASE VIRUS STRAINS ISOLATED FROM INFECTED CHICKENS

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Newcastle disease virus (NDV) causes a highly contagious disease of poultry. NDV is a member of the genus *Avulavirus* of the *Paramoxyviridae* family [1]. This is an enveloped virus, having a non-segmented single stranded linear negative RNA genome. The genome of NDV encodes six major structural and two non-structural proteins [2, 3]. The fusion protein, along with hemagglutinin-neuraminidase, serves as the target for the host immune response. Different strains have different number of amino acids in the cleavage site of the fusion protein [4].

NDV strains have been grouped into pathotypes based on the clinical signs produced in infected chickens. There are lentogenic, mesogenic and velogenic pathotypes. Velogenic are highly pathogenic and lead to haemorrhagic intestinal lesions and neural sings. The mortality may be up to 90-100%. Typical strains: GBTexas, NYParrot 70181, Herts 33\56. Mesogenic strains cause respiratory sings, occasional nervous sings, but low mortality. Typical strains: Beach, Beaudett, Roakin. Lentogenic or respiratory strains can cause only mild or subclinical respiratory infection (Lasota, F, Hitcher B1). There are also so called asymptomatic strains. They can lead to a subclinical enteric infection (QeensiandV4, V4HR) [1, 5].

According to the latest data economic losses due to Newcastle disease are very high in case of velogenic strains which cause 90 - 100% mortality of infected birds. Also production levels of vaccinated flocks are 20 – 60% reduced and lots of finance are spent for treatment and prevention of the disease [6, 7]. The risk of outbreak of Newcastle disease in Ukraine is very high because the disease occurs around the world every year. The virus can be brought to Ukraine with wild migratory birds, infected poultry products and the persons who had contact with sick poultry, etc.

That's why the aim of this work was to establish the presence of NDV in pathological material taken from chickens with signs of Newcastle disease, using PCR and identify pathotype of detected NDV strains with the help of restriction analysis.

For the research 16 samples of pathological material (tracheas, lungs, intestines - the main targets for viral replication) were taken.

Total RNA was extracted with silica-based method (Ribo-sorb, Amplisens). Then reverse transcription was performed with the use of random primers (Reverta-L, Amplisens). PCR was performed in two stages using two pairs of primers complementary to the sequence of F gene [7, 8]. In the first phase, cDNA was a matrix for reaction, size of amplicon was 356 b.p. To increase the sensitivity of the method we used a second pair of primers that flanked 216b.p sequence within the first amplicon sequence. The visualization of the reaction products was done by electrophoresis in 1.5% agarose gel.

For the restriction analysis we used 2 restriction enzymes BglI and HhaI. These two restriction sites can determine the NDV pathotype. Mesogenic strains are characterized by HhaI restriction site, lentogenic strains have both BglI and HhaI restriction sites. Velogenic strains do not have these restriction sites [9].

During the study virus was detected in 5 of the 16 tested samples.

BglI restriction site was detected in two samples, restriction site HhaI – in 4 samples. For one sample no restriction sites were shown.

Therefore, due to the combination of restriction sites, two NDV isolates belonged to lentogenic, two to mezogenic and one of isolate had restriction profile typical for velogenic strains.

The presence of velogenic strains is a major threat for poultry industry, so this research should be continued to establish the spread of NDV strains in Ukraine for feather introduction of measures to control the disease.

1. Alexander D.J. Newcastle Disease and Other Paramyxovirus Infections / Calnek B. W. [et al.]. // Iowa State University Press.- 1997. –P. 541-569.
2. Peeters B.P. Genome replication of Newcastle disease virus / Gruijthuijsen Y.K. [et al.]. // Arch Virol. -2000. –Vol.145. –P. 1829-1845.
3. McGinnes L.W. Inhibition of Receptor Binding Stabilizes Newcastle Disease Virus HN and F Protein-Containing Complexes / Morrison T.G. // J Virol. –2006. –Vol.80. –P. 2894–2903.
4. Ballagi-Pordany A. Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from F gene // Archivesof Virology. -1996. Vol.141. –P. 243-261.
5. Asplin F.D. Immunisation against Newcastle disease with a virus of low virulence (Strain F) and observations on subclinical infection in partially resistant fowls // Vet Rec. - 1952. – Vol.64. –P.245—249.
6. Alexander D.J. Newcastle disease virus pathotypes // Avian Pathology - 1974. –Vol. 4. –P. 269-278.
7. Bangl J. Isolation and detection of Newcastle Disease Virus from field outbreaks in broiler and layer chickens by reverse transcription polymerase chain reaction // Haque M. H. [et al.]. // Vet. Med. - 2010. – Vol. 8. – P.38-45.
8. Shahina Kalim Development and in vitro characteriasation of a bivalent DNA containing HN and F genes of velogenic Newcastle disease virus / Barkha Ratta // Uttara Chaturvedi, Indian Journal of Experimental Biology. -2011. –Vol.49. –P. 140.
9. Nanthakumar T. Pathotyping of Newcastle disease viruses by RT-PCR and restriction enzyme analysis / Nanthakumar, T [et al.]. // Veterinary Research Communica-tions. -2000. –Vol.24(3).-P.275-286.

# SPREADING AND PHYLOGENETIC ANALYSIS OF FULL-GENOME SEQUENSES OF UKRAINIAN ISOLATES OF CIRCOVIRUS TYPE 2

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Circovirus-associated syndromes of pigs (CASP) are among the most typical in many countries of the world, especially in Europe. For establishing a dependency between occurrence of the virus and animal age, and to elucidate geographical spread of this virus in Ukraine, we conducted tests of viral DNA isolated from material combined on the base of sampling locations and age of animals. In this part work we used a pair of primers Cir_orf2_F and Cir_orf2_R flanking a specific 502 bp portion of the Cap gene. Electrophoretic separation of PCR products showed specific amplicons in 57 studied samples from animals of every age group in different regions of Ukraine. Therefore, Circovirus type 2 was detected in 57 (of 108) samples from animals of various age groups collected from farms in 16 (of 22) studied regions of Ukraine. Interestingly, all samples were Circovirus type 2-positive in case of 6 regions: Kharkiv, Kyrovohrad, Volyn, Zakarpattya, Kherson and Ternopil. For the other 5 regions (Donetsk, Zaporizzhya, Kyiv, Odessa and Sumy), more than 50% of samples were virus-positive. Circovirus type 2 was detected in less than 50% of samples collected in Vinnytsya, Dnipropetrivsk, Cherkassy, Chernihiv and Poltava regions. Finally, this pathogen has not been found in samples collected from animals in Chenivtsy, Khmelnytsk, Rivne, Mykolayiv, Lugansk or Ivano-Frankivsk regions.

Phylogenetic analysis of full-genome sequenses of Ukrainian isolates of Circovirus type 2 (NJ method) showed their significant heterogeneity and indicated their attribution to 3 major groups: 2a, 2b and 2c.

# SPECIFIC PREVENTION OF PORCINE EPIDEMIC DIARRHEA VIRUS

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First cases of epidemic diarrhea in pigs (PED) in Ukraine were recorded in 2014. Disease demonstration was characterized by profuse diarrhea in pigs of all groups and a high mortality rate (100%) in lactating piglets [1].

Nowadays in the world there are no specific or non-specific prevention, which can effectively control the virus and prevent disease. Therefore, the analysis of the situation and development of PED preventing means is an actual direction of infectious diseases studying [2].

The aim of our study is to evaluate the effectiveness of specific methods of immunization on the PED-positive farms.

For specific prevention of disease in farms were used two methods:

1. The method of reverse feeding of pathological material (bowel) from killed or dead pigs containing the virus within 104. Material was fed twice for 4 and 2 weeks before the planned farrowing.

2. The method of vaccination of sows with inactivated autologous vaccine, designed to prevent pigs from PEDV. Vaccination held twice, 5 and 2 weeks before the planned farrowing. Dose of the vaccine 2ml, was entered intramuscularly.

Detection of the virus RNA and determination of its amount were spent by PCR. The nature of the immune response was determined by ELISA. The materials for experiment were selected from the 20 heads of sows for each method.

At the beginning of the experiment were received a serum from sows to determine the level of protective antibodies against the virus. Titles ranged from 0,2 to 1,2 (cut off 0,4, the test system Biovet). Also set the amount of virus that stood out with the faeces, the level was 103 in 1g.

Method 1. In sows before farrowing were identified different titres of serum antibodies to the PED virus. It was ranged from 0.6 to 2.2. In 23.7% of piglets from sows were observed clinical signs of diarrhea at the day 3 after birth. the amount of virus from faeces were 103 of virus RNA in 1 g of feces.

Method 2. In sows before farrowing virus titres antibodies against PED varied within 2,2-2,5. The minimum level of antibodies marked in gilts it were 1,6-19. Clinical signs of the disease recorded on day 5 after birth of piglets with body weight less than 1kg of gilts litter. Number of virus RNA that was isolated from the feces was 102 in 1g of feces.

The results show that using of the method of reverse feeding does not provide a stable level of antibodies in sows before farrowing, the using of this method leads to increasing the number of virus, that released into the environment.

Using the vaccines provide a stable antibody titer and does not affect on the amount of virus isolated from feces.

These results revealed that the method of vaccination with autologous vaccines against PED is more efficient and safer than the method of reverse feeding. However, it should to further explore the nature of developments of antibodies in gilts and IgA levels in sows colostrum immediately after farrowing.

1. Dastjerdi Akbar Porcine Epidemic Diarrhea Virus among Farmed Pigs, Ukraine / Dastjerdi A., Carr J., J. E. Richard [et al.] // EID J.- Dec.2015.-№10

2. Kwonil Jung Porcine epidemic diarrhea virus infection: Etiology, epidemiology, pathogenesis and immunoprophylaxis / Jung K., Saif L. J. // Vet. J.- May.2015.-№2.-P.134-143

# PRRSV: DYNAMIC OF SPREADING IN UKRAINE, MOLECULAR-BIOLOGICAL CHARACTERISTICS OF UKRAINIAN ISOLATES

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Porcine reproductive and respiratory syndrome virus are currently prevalent in most pork producing countries. [1, 2]. The fast development of pork industry and insufficient management are the most actual factors which can cause the fast spread of PRRSV in Ukraine.

The uniqueness of PRRS virus is the highest level of genetic variability among all RNA animal viruses. Another ability of this pathogen is its long-term persistence. PRRSV virus is able to multiply in macrophages and dendritic cells causing immunosuppression. This causes immunodeficiency state of animals and increases their susceptibility to opportunistic pathogens.

The PRRSV situation in Ukraine wasn't studied enough that is why our aim was to figure out the spread of the porcine reproductive and respiratory syndrome virus in Ukraine. Considering great variability of PRRSV strains and lack of described strains in Ukraine one of the objective of the investigation was to to implement a method of detecting the virus genome and sequencing in order to determine nucleotide sequence of hypervariable region of ORF5 gene of different PRRSV isolates circulating in farms of Ukraine. For analyzing the epizootic situation in farms and to stop spreading PRRSV through it the use of ELISA is required. The critical points for usage of this method are timing of sampling and the usage of sensitive ELISA test-kit.

So the main aim of our study was to investigate molecular-biological characteristics of the porcine reproductive respiratory syndrome virus, circulating in Ukraine.

During 2005–2014 in 24 regions of Ukraine and Crimean Peninsula , 96437 blood samples were obtained from animals of 252 herds. The sampling of blood serum from animals of different age groups was carried out. For this study serum samples were collected from animals from Crimea Penincial and 24 regions of Ukraine. Sera were tested for the detection of PRRSV antibodies by the Herd Check PRRS commercial ELISA kit (IDEXX Herd Check PRRS X2 ELISA in 2005-2009 years and then IDEXX Herd Check PRRS X3 ELISA, USA). The presence of maternal antibodies to PRRSV and the fact of their decrease prior to 9 weeks of animal's life were also taken into account [6, 11]. To obtain reliable results, serum samples were tested from animals of different age groups of one farm [12] in an amount not less than 12 [6].

For the detection of PRRSV in the pathological material from the animal we developed PCR test-kit for the detection of conservative region of PRRSV genome – ORF7. This test kit were implemented for usage in diagnostics of PRRSV. We need to emphasize that this test-kit cant be used for genetic analysis as it is not informative. The variability is quite rare to be detected in ORF7 region of genome. For this reason we obtained PCR amplification of hyper variable region of ORF5 gene with specific primers. The nucleotide sequence of the PRRSV was determined for 8 Ukrainian PRRSV isolates.

The result of sequence was compared with strains from Eastern and Western Europe, as well as vaccine strains to establish the relationship

between them and built a phylogenetic tree using MEGA 6. All isolates were characterized as PRRSV European genotype and no North-American strains were detected. The results of the study have shown that 4 isolates formed a separate group, and showed the highest degree of homology with isolates from Russia and Italy. The sample №3 was closely related with Spanish strains. Isolate №4 has the highest homology with Italian and Belarusian isolates. This isolate could arise as a result of simultaneous infection of two PRRSV strains in the body of one animal. Phylogenetic position isolate №2 was closely related to strains Spanish and Italy strains of PRRSV.

For the first estimation of epizootic situation with porcine reproductive and respiratory syndrome virus ( PRRSV) on the farm we propose to analyze blood serum from 24-weeks old animals using ELISA. The method of the serological profile constructing (based on ELISA) is the most sufficient method to establish informative dynamics of infection associated with the PRRSV. We showed the optimal age group for the screening of blood serum: 4, 7, 10, 15, 18 and 24 weeks of age, usage of which will be enough not only to determinate the epizootic situation on the farm but to establish the time of infection occure in order to prevent it.

In this study we have reported fast spread of PRRSV in Ukraine as we detected PRRSV positive serum samples in 19 regions in 2014 year. In fact in 2005 we found PRRSV positive animals only in 2 regions of Ukraine. All our data are regarded as an evidence of the fast spreading PRRSV through Ukraine. Also we implement PCR diagnostics of PRRSV and determined nucleotide sequence of hypervariable region of ORF5 gene of different PRRSV isolates circulating in Ukrainian farms and compare obtained sequences with previously characterized and available in GenBank. Also we showed the optimal date of serum sampling for constraction an informative serological profile in diagnosis of PRRSV.


1. Van Breedam W., Costers S., Vanhee M. et. al. Porcine reproductive and respiratory syndrome virus (PRRSV) – specific mAbs: supporting diagnostics and providing new insights into the antigenic properties of the virus // Veterinary Immunology and Immunopathology.-2011.-P.1-12.
2. Stadejek T., Oleksiewicz M.B, Scherbakov A.V. et al. Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: nucleocapsid characteristics and geographical distrubition in Europe // Arch.Virol. – 2008.-Vol.153. – P.1479-1488.
3. Stadejek T., Balka G., Chabros K., Lipej Z., Novosel D., Stankevicius A. Genetic diversity of European genotype PRRS in Central an Eastern Europe – an update // 21st international pig veterinary society congress. – Vancuver, 2010.

4. Ivashchenko O.A. The spread of porcine reproductive and respiratory syndrome virus in Ukraine.//Вісник Київського національного університету імені Тараса Шевченка,К. – 2012. - Т.62. – С.9-11.

# BIOLOGICAL CHARACTERISTICS OF INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS STRAIN "BM"

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Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis (IBR/IPV), caused by bovine herpesvirus 1 (BoHV-1), is a disease of domestic and wild cattle. The virus is distributed world-wide, but has been eradicated from Austria, Denmark, Finland, Sweden, Italy (Province of Bolzano), Switzerland, Norway and parts of Germany (the 'Oberfranken' and 'Oberpfalz' districts of Bavaria). Control programmes are running in several other countries, for example in Germany and Italy [1, 2].

The uniqueness of IBR virus is in possibility of integration of virus genome into the genome of the host cell and thereby long-term persistence. IBR virus is able to multiply in monocytes, neutrophils and macrophages, causing immunosuppression. This causes immunodeficiency state of animals and increases their susceptibility to opportunistic pathogens, which in turn prevents adequate and effective immune response after administration of the vaccine [2, 3, 4]. Isolation of new strains of viruses that circulate in certain areas is always actual topic, especially in creation of vaccine [5].

The aim of our study was to investigate biological characteristics of the infectious bovine rhinotracheitis virus, which was isolated from a calf in one of the farms in Ukraine.

Pathological material for virus isolation was collected from killed for diagnostic purposes calf, whose observed symptoms were characteristic for infectious bovine rhinotracheitis. Were selected following samples: swabs from the mucosa of the nasal cavity, nasal septum pieces, choanae and larynx. Preparation of the samples was carried out by grinding and introducing solutions of antibiotics. Virus isolation was performed by

infecting cell cultures MDBK, simultaneously performed PCR analysis to confirm the presence of the IBR virus in samples.

Cell culture was grown in culture flask 25cmI area for 1-2 days to formation of solid monolayer of cells. After a preliminary laundering of cells monolayer with Hanks solution, in culture flask contributed prepared sample. For the contact of a virus with cells, flask were moved in an incubator for 1 hour at 37 ±0,5°C. After was added supportive culture medium «DMEM + RPMI» in 1:1 ratio without serum.

Infected cells were observed until appearance of virus cytopathic effect (CPE) for 96 hours using inverted microscope. If, during observation period, CPE of a virus is not observed, culture was frozen, and conducted next passage.

Level of a virus accumulation in the cell suspension was determined by titration of the material obtained in cell culture and calculated titer of infectious activity of the material by the method of Reed and Muench [6].

An examination of farm detected cases of the disease in calves of 6-9 months age. Clinical signs were expressed quite intensively: fever, depression, inflammation of the upper respiratory tract, cough, serous leakage from the nasal cavity and eyes, loss of appetite. The clinical manifestation of the disease was observed for 2 weeks, during this period calves were not treated.

At calf autopsy were observed changes typical for infectious bovine rhinotracheitis. In nasal cavity was observed in the front third of the ventral shells, ventral nasal passages and nasal septum. Mucosa was strongly hyperemic, dark red, swollen, filled with point hemorrhages, on mucosal surfaces accumulation of serous fluid. Mucous membrane of larynx with point hemorrhages.

At the same time were taken samples of affected tissues and swab from mucous membrane of nasal cavity for virological research. Indication of the virus and studying of his biological characteristics were performed in cell culture MDBK. Results of virus isolation are presented in Table 1.

**Table 1**

**Virological studies of material, (M±m; n=3-5)**

| № passage | Name of material | Manifestation of CPE | Incubation time, hours | Infectious activity, lg $TCD_{50}/cm^3$ | PCR result |
|---|---|---|---|---|---|
| 1 | Swabs from the mucosa | - | 96 | -- | + |
| | Tissue samples | + | 96 | -- | + |
| 2 | Swabs from the mucosa | - | 96 | -- | + |

| | | | | | |
|---|---|---|---|---|---|
| | Tissue samples | ++ | 96 | -- | + |
| 3 | Swabs from the mucosa | + | 96 | 2,5 ±0,08 | + |
| | Tissue samples | +++ | 48 | 5,56 ±0,04 | + |
| 4 | Swabs from the mucosa | ++ | 96 | 3,5 ±0,08 | + |
| | Tissue samples | ++++ | 24 | 6,27 ±0,13 | + |
| 5 | Swabs from the mucosa | ++ | 96 | 3,62 ±0,04 | + |
| | Tissue samples | ++++ | 24 | 6,66 ±0,10 | + |

Data in Table 1 show that IBR virus in swabs from the mucosa which was inoculated in cell culture showed cytopathic effect on the 3rd passage after 96 hours of incubation, and by the 5th passage for 96 hours of incubation only slightly increased cytopathic manifestation. Isolation of IBR virus from tissue samples was accompanied by a more intense manifestation of CPE of virus in cell culture, achieving maximum destruction of the cells on the 5th passage at the 24 hour of incubation. The titer of infectious activity of the virus while on the 5th passage was 6,66 ±0,10 lg $TCD_{50}/cm^3$.

IBR virus isolated from infected tissue samples from calf showed the highest accumulation and intensity of virus CPE manifestation . Virus introduced into work for determining of its biological and cultural properties. To the isolated strain of infectious bovine rhinotracheitis was given name "BM".

1. OIE Terrestrial Manual 2016 / Chapter 2.4.12. – Infectious bovine rhinotracheitis / infectious pustular vulvovaginitis - Retrieved from: http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/
2. Infectious bovine rhinotracheitis / A.J. Samujlenko et al. // Infectious pathology of animals. – 2006. – Vol.1. – P. 662–667.
3. Glotov A.G. Infectious bovine rhinotracheitis / A.G. Glotov, A.F. Shuljak, T.I. Glotov, A.N. Sergeev. - Novosibirsk, 2006. – 196p.
4. Kostyrkin Y.A. Measures of prevention and fight against infectious bovine rhinotracheitis - infectious pustular vulvovaginitis cattle in a dairy cattle / Y.A. Kostyrkin, V.A. Mischenko, J.V. Nesterenko et al. // Proc. Federal Centre for Animal Health. - Vladimir, 2005. - Vol.3. - P. 171-183.
5. Bashir S., Dar A. M., Bhat A. A., Bukhari S., Hussain T. Isolation of infectious bovine rhinotracheitis virus in cell culture (a case study) // Journal of Cell and Tissue Research 15.2, 2015, P. 5063-5066.
6. Reed L.J. A simple method of estimating fifty percent endpoints / L.J. Reed, H. Muench // Am. J. Hygiene, 27, 1938, P. 493-497.

# CLINICAL SIGNS IN DIAGNOSTIC OF CIRCOVIRUS INFECTION IN PIGS

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Porcine circovirus is obe of the most studied pathogens among those that are the most actual during the last 15 years. However, in Ukraine the disease associated with porcine circovirus (PCVAD) become signs of a pandemic. Detection of specific antibodies in the blood serum of pigs shows that circovirus infection of pigs (PCVD) is widespread in the world, regardless of the status of PCV2 farms [2].

Efficiency of histological, immunohistochemical and PCR methods for the diagnosis of circovirus infection in pigs has been proven many times. However, clinical signs PCVAD imitate many other diseases (classical swine fever, transmissible gastroenteritis, porcine parvovirus, pseudorabies (Aujeszky disease), leptospirosis et al.), which complicates diagnosis of the disease in the early stages of infection among livestock. If experts of a farm think there is no presence PCV2, they will not do additional diagnostic tests. Typically, diagnosis PCVAD occurs when the infection has become total, chronic course. Late diagnosis, lack of treatment and high price vaccines contribute to the formation of stationary fire PCVD. Therefore, the preliminary diagnosis based on clinical signs and pathological changes in animals suffering from PCV2, is the primary in fight PCVD PCVAD.

PCV2 causes the development of immunodeficiency, so animals are usually infected by several agents, including parvovirus pigs, swine reproductive and respiratory syndrome virus, Swine Hepatitis E virus, *Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis, Staphilococcus spp., Streptococcus spp*. [2, 5]. The most studied is the association of swine circovirus virus reproductive and respiratory syndrome pigs and porcine parvovirus [1].

The main targets for PCV2 in the neonatal period are cardiomyocytes, hepatocytes and macrophages. In postnatal virus replicates well in the cells of the lymphatic system, heart, lungs, liver, spleen, kidneys and brain [3]. So symptoms of circovirus infection of pigs vary on clinical grounds. For postweaning multisystemic wasting syndrome - PMWS

(PCV2-SD) characteristic is the development of acquired immunodeficiency. This syndrome occurs most frequently. Lymphocyte depletion of lymphoid tissue, changes subpopulations in peripheral blood mononuclear cells, expression changes of cytokines are registered. Clinically it is manifested in typical weight loss among pigs aged 8-18 weeks. Muscle atrophy, cardiomyopathy and pulmonary pathology leads to choke, anemia, pale mucous membranes. Sometimes there is jaundice and sporadic diarrhea. Inguinal lymph nodes are enlarged. As a result of the temperature subfibrylnoyi animals are suppressed. The mortality rate among infected pigs PCV2-SD ranges from 5-50%. Body weights gain is less than normal, becoming ready for being sold increases [1, 2]. Three times increased in lymph nodes, thymus atrophy, infarction of the spleen, diffuse edema of the lungs, liver jaundice, enlarged kidneys with areas of infiltration of lymphocytes, stomach ulcers, multifocal myocarditis are recorded during the autopsy. Almost in all organs infiltration of lymphocytes is detected.

The course PCVD in the form of subclinical infections (PCV2-SI) is characterized with invisible clinical signs. Low body weight gain amonf animals of feeding group is the only sign. Experts of farms can link it with poor quality food, sanitary conditions of the premises, etc. swine genetics. Typically PCV2-SI is recorded in disadvantaged farms where PCVD affects pigs for years and is never diagnosed.

The course of PCVD in the form of disease of the reproductive system (PCV2-RD) is characterized with abortion in the third trimester of gestation and birth of non-viable offspring. This syndrome is characterized by the absence of other clinical signs in sows. Veterinary experts in this case may suspect brucellosis, leptospirosis, mycoplasmosis, mycotoxicoses, PRRS and swine parvovirus.

The course in the form of PCVD dermatonefropatychnoho syndrome (PDNS) should also be considered as one of the clinical manifestations of PCVD. PDNS is registered among pigs aged 2-6 months. Pigs older than 8 months it is almost undetectable. This syndrome refers to the of hypersensitivity type III. Typical for PDNS is acute course with signs of anorexia, depression, hypotension, or 40,5 - 41$^{o}$C temperature, necrotizing vasculitis. On the skin in the hind limbs and perineum appear erythema, which is then covered with a thin scab and disappear on its own. These symptoms are sometimes confused with signs of acute course of erysipelas.

Respiratory disease complex (PRDC) is considered as a separate syndrome PCVAD. It is a multifactorial disease that affects pigs 12-24 weeks aged [6]. It is characterized with fever, cough, nasal leakage, acute

pulmonary edema, weight loss. It can be confused with pasteurellosis, haemophilosis pleuropneumonia and other diseases.

According the notification of clinical and pathological signs PCVD we examined the number of pigs on the farm that specializes in young animals received and fattening. The farm has reproductive herd. Pigs were kept in suboptimal health conditions (lack of ventilation, high content of ammonia in the air, low temperature in the rooms). Regular diagnostic and preventive measures were not taken. Specialists farms concerned about the low increase in body weight, but they did not think of circovirus infection pigs. We divided all pigs into three groups. The first group - adult sows, the second group - pigs aged 0-28 days, the third group - pigs aged between 35 - 180 days. All animals were clinically examined. Animals of the first group did not have clinical signs specific to PCVD. Animals of the second group were characterized with heterogeneity of group, weight of piglets at birth ranged from 0.9 to 1.3 kg. Animals were behind in growth, development, mortality ranged from 10%. Diarrhea and cough were not detected. The third group of animals was characterized with strong heterogeneity of herds and low growth. Testing revealed a lag in growth and development, depression, pale mucous membranes, increasing the inguinal, submaxillary, retropharyngeal lymph nodes. Autopsy revealed: fibrinous pleuropneumonia, myocarditis, filling with blood of spleen and liver, enlarged kidneys, increased all the lymph nodes, especially inguinal and ripples. Signs of generalized staph infection were recorded with one pig. Using PCR analysis confirmed PCV2.


1. AllanG.M., Ellis J.A. Porcine circoviruses: a review. // J. Vet Diagn Invest. -2000;-12, P. 3–14.
2. Segalйs J, Allan G.M., Domingo M. Porcine circovirus diseases // Anim Health Res Rev. -2005, -Dec, -6(2), -P. 119-42.
3. Romeo E, Sanchez R.E. Jr., Meerts P., Nauwynck H.J., Pensaert M.B. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life // Vet. Microbiol. - 2003, - Aug, -№ 29;95(1-2), -P.15-25.
4. Opriessnig T., Langohr I. Current state of knowledge on porcine circovirus type 2-associated lesions // Vet Pathol.- 2013, - Jan, - 50(1), -P. 23-38.
5. Yifei Yang, Ruihan Shi, Ruiping She, Jingjing Mao, Yue Zhao, Fang Du, Can Liu, Jianchai Liu, Minheng Cheng, Rining Zhu, Wei Li, Xiaoyang Wang and Majid Hussain Soomro. Fatal disease associated with Swine *Hepatitis E virus* and *Porcine circovirus 2* co-infection in four weaned pigs in China // *BMC Veterinary Research,* - 2015, - 11, -P.77.

6. Ivica Gjurovski, Branko Angelovski, Toni Dovenski, Dine Mitrov. Diagnostic Characteristics of Circovirus Infection in Pigs //Macedonian Veterinary Review, Volume 38, Issue 1 (Mar 2015), - P. 73-78.

# GENETIC CHARACTERIZATION OF INFECTIOUS BURSAL DISEASE VIRUS ISOLATES IN UKRAINE

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Infectious bursal disease virus (IBDV) belongs to the *Birnaviridae* family *Avibirnavirus* genus. It has a non-enveloped, icosahedral capsid. Viral genome consists of two segments of double-stranded RNA. Virus replicates in immature IgM+ B-cells residing in the bursa of Fabricius of young chickens and causes infectious bursal disease or Gumboro disease. Two serotypes of the virus have been described. Serotype 1 IBDV strains are pathogenic to chickens, whereas serotype 2 strains are non-pathogenic [3]. Serotype 1 IBDV isolates comprise the variant, classical virulent (cvIBDV) and very virulent (vvIBDV) strains, which greatly differ in their pathogenicity to chickens. VvIBDV strains were detected in Europe in 1986 and caused 70% mortality in susceptible chickens. These strains still cause great economic impact in poultry industry worldwide [1,2]. VvIBDV strains have been characterized in many countries, but there were no publications about these strains in Ukraine. Most of the modern research has been focused on the VP2 protein as it is the primary antigen determinant and major structural protein [5]. VP2 gene contains special hypervariable region (VP2 HRV). Mutations in this region lead to emergence of the new antigenically different IBDV strains [4].

Considering great variety and variability of IBDV strains and lack of described strains in Ukraine the objective of the investigation was to determine nucleotide sequence of hypervariable region of VP2 gene of different IBDV isolates circulating in poultry farms in Ukraine and compare

obtained sequences with previously characterized and available in GenBank.

For this research bursa samples were collected from infected chickens at the age of 24 - 44 days. RNA was extracted with silica-based method (Ribo-sorb, Amplisens). Reverse transcription was performed using a set of random primers (Reverta-L, Amplisens). PCR amplification of hypervariable region of VP2 gene was carried out with specific primers. 552 bp PCR product was visualized in 1,5 agarose gel. Amplicons were separated from reaction components using the Thermo Scientific GeneJET Gel Extraction Kit. Purified amplicons were sequenced using forvard primer by Institute of Molecular Biology and Genetics (NAS, Ukraine). Nucleotide alignment was performed using ClustalW instrument, within Mega 6, with the phylogenetic analysis using the neighbour-joining method.

The nucleotide sequence of the VP2 HVR was determined for 16 Ukrainian IBDV isolates. 8 isolates were characterized as very virulent, and 8 as classical virulent strains (fig.1). Ukrainian vvIBDV strains were clustered together with very virulent strains from other counties like: United Kingdom, UK661 (AJ878898); Egypt, K406/89 (AF159218); China, HK46 (AF051838); Netherlands, 1986 (Z25482); Spain, SP/31/02 (AY770593). Detected isolates were also genetically related to attenuated vvIBDV strains MB and MB/3. It is assumed that vaccines based on these strains will provide the best protection against very virulent strains.

Very virulent isolates formed two subgroups (VV-1 and VV-2) which indicates on different origin of these strains. Nucleotide identity among vvIBDV strains detected in Ukraine varied from 99,8% (between strains 934 and 964) to 94,4% (between strains 1517 and 964).

Classical strains 760_45_5, 691_24 and 1147 were closely related with referent strains CU-1 (AF362771), Croatia.Cro-Pa/98 (EU184689) and classical attenuated vaccine strains.

Isolates 58, 43_1943, 38_1943, 2045 and 1853 formed separate group with vaccine strains V877 and MB/5. This data indicates that these strains were isolated from chickens that were previously vaccinated with IBDV vaccines that contain V877 or MB/5 strain. Amino acid sequence analyses is needed for feather investigation.

In this study we have reported vvIBDV isolates circulating in Ukraine. Ukrainian vvIBDVs cluster phylogenetically with previously characterized vvIBDVs from other countries and attenuated intermediate plus or “hot” vaccine strains. Classical Ukrainian strains were closely related to intermediate attenuated vaccine strains. Information from this study could be used to guide IBDV vaccine selection in Ukraine.

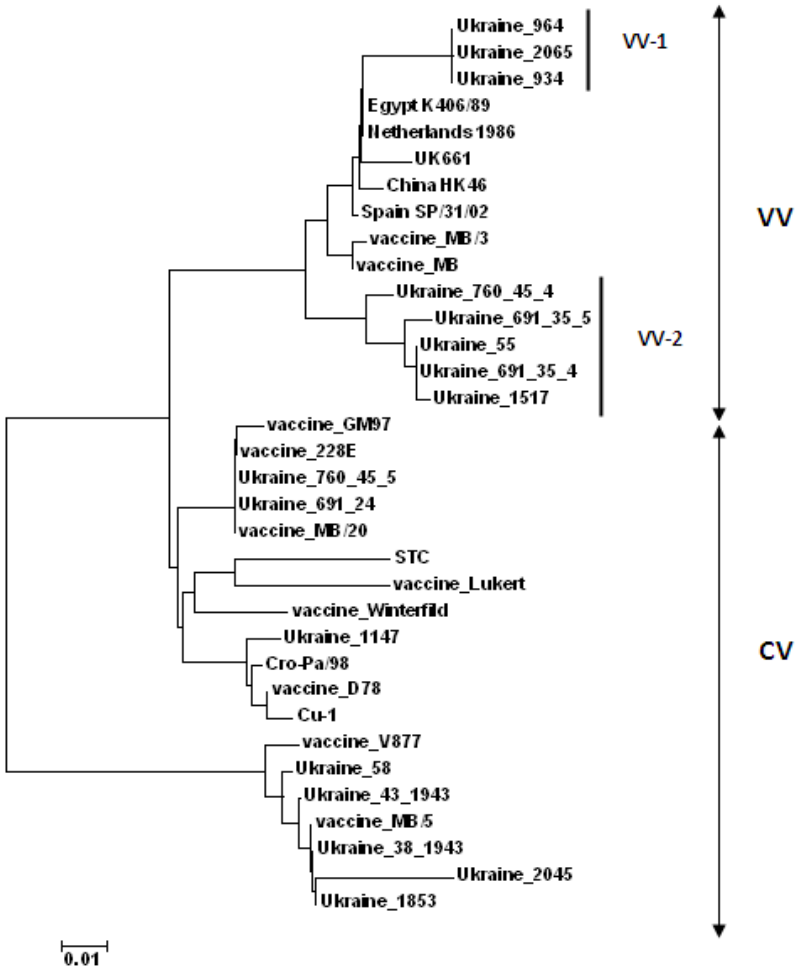


**Figure 1.** Phylogenetic analysis of the VP2 hypervariable coding sequence of 34 IBDV isolates (neighbour-joining method, Mega 6 software).

1. Jackwood D.J., Sommer-Wagner S.E., Crossley B.M. Identification and pathogenicity of a natural reassortant between a very virulent serotype 1 infectious bursal disease virus (IBDV) and a serotype 2 IBDV // Virology. – 2011. – Vol. 420, № 2. – P. 98 – 105.

2. Jenberiea S., Lynchb S.E., Kebedea F. Genetic characterisation of infectious bursal disease virus isolates in Ethiopia // Acta Tropica. – 2014., Vol. 30, P. 39–43.
3. Kegne T., Chanie M. Review on the Incidence and Pathology of Infectious Bursal Disease // British Journal of Poultry Sciences. – 2014. – Vol. 3, № 3. – P. 68 – 77.
4. Letzel T., Coulibaly F., Rey F.A. Molecular and structural bases for the antigenicity of VP2 of infectious bursal disease virus // J Virol. – 2007. – Vol. 81, № 23. – P.12827 – 12835.
5. Mawgod S.A., Arafa A.S., Hussein H.A. Molecular genotyping of the infectious bursal disease virus (IBDV) isolated from Broiler Flocks in Egypt // International Journal of Veterinary Science and Medicine. – 2014. – Vol. 2, № 1. – P. 46 – 52.

# MOLECULAR IDENTIFICATION OF SPRING VIRAEMIA OF CARP VIRUS ISOLATED IN UKRAINE

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Spring viraemia of carp (SVC) is a viral disease caused by Spring viraemia of carp virus (SVCV) which is classified as a member of the family *Rhabdoviridae*, belonging to the genus *Vesiculovirus*. The genome of SVCV is a linear single-stranded negative that encodes five structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and viral RNA-dependent RNA polymerase (L) in the order: 39-N-P-M-G-L-59.

The International Epizootic Bureau (OIE) classified SVC as an especially dangerous disease of cultured fish. The study of SVCV causing this disease in Ukraine is of special interest because Cyprinids are traditional objects of national aquaculture and the annual carp yield of

stocked species amounts to 20,000 tons. Carp mortality (mainly that of fingerlings) usually amounts to 30–40%, but sometimes can reach up to 70%. In this study we presents the results of PCR detection and the nucleotide sequence analysis of the Ukrainian strains of SVCV, isolated from carps in Ukraine.

Genomic viral RNA extraction and cDNA synthesis were conducted using commercial kits. Amplification of viral ssRNA fragment targeting the glycoprotein gene G has been carried out using the virus specific primers and two-step PCR. Sequencing was performed on a 3130 Genetic Analyzer (Applied Biosystems). The obtained sequences were analyzed using BLASTN and MEGA software.

The size of PCR products of Ukrainian isolates of SVCV was 606 base pairs. It should be noted that after the first round of PCR, products were not visible on agarose gels. To avoid such problems, we used the semi-nested assay using the second round of PCR. The specificity of amplified PCR products was verified by the sequencing analysis. The results of sequencing showed that amplified PCR products were identical to fragments of the SVCV glycoprotein gene G deposited in the GenBank by other researchers. The phylogenetic analysis of the glycoprotein gene G based on the comparison of Ukrainian isolates of SVCV with other available sequences revealed a close relationship of Ukrainian isolates of SVCV with sequences that represent the Fijan strain.

Today the molecular epidemiology is an important instrument for the disease investigation and control due to its ability to reveal the possible sources of infection. The molecular characterization of viral pathogen allows to trace phylogenetic relationships of the virus and to determine the evolutionary history. In addition the development of highly sensitive methods for diagnostics based on polymerase chain reaction (PCR) helps researchers to reveal mutations and acquisition of new properties by isolates that spread in certain areas.

# ESTABLISHMENT, CHARACTERISTICS AND VIROLOGICAL APPLICATION OF A RED-EARED SLIDER TURTLE *(TRACHEMYS SCRIPTA)* CELL CULTURE

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In the last decades, due to changing environments, increasing tourism, international traffic and trade in exotic animals, concern about the risks of spreading pathogens remains high among epidemiologists, virologists and veterinary authorities. Amphibians and reptiles ranked third in the list of the most traded animals. The potential risk of the movement of infected, non-native "exotic" animals is spreading invasive species and novel pathogens that may be harmful for local wild animal populations, livestock, human and often having economically and ecologically disastrous consequences. Approximately 200 recognised human diseases are linked to animals. Known as 'zoonoses', about 40 of these pathologies are associated with amphibians and reptiles alone. A survey of 1,410 human diseases, however, found that 61% may have a zoonotic origin. Wildlife markets have been highlighted as an especially high-risk infection hub due to species diversity, poor hygiene and stressful and cramped conditions that facilitate microbial transfer. Many cases of zoonotic disease superficially resemble common illnesses, such as gastrointestinal disturbances and 'flu-like' conditions, and thus may be misdiagnosed. Although important and of rapidly growing concern, the prevalence of zoonotic disease in the human population is at present challenging to quantify. Some results obtained in isolate of dangerous pathogens indicate that reptilians are the reservoirs of many viruses from different Families which may have zoonotic potential, such as arbovirus group (Togaviruses, Flaviviruses, Rhabdoviruses, and Bunyaviruses) transmitted by arthropods and causing infections of humans as well as of other mammals and birds. In the past decade, outbreaks of infection with West Nile virus in human populations and in farmed alligators in the USA has seen the research emphasis placed on the issue of reptiles, being susceptible to, and reservoirs for, serious zoonotic disease. Besides, constantly growing number of exotic animals can pose a serious threat to native fauna. The release of pets into the wild have the capacity to

introduce novel and harmful pathogens to indigenous wildlife. For example, in the USA, the release of pet tortoises is what most likely led to widespread introduction of 'upper respiratory tract disease syndrome' (URDS) which killed 79% of free-living tortoises. Also, the international trade in amphibians may have been responsible for the spread of the Ranavirus, causing ranavirosis. This disease has been associated with the observed global decline and extinction of amphibian species and was listed as a World Organisation for Animal Health (OIE) notifiable disease in 2008. A list of diseases of reptiles has been included by OIE's experts of the Working Group on wildlife diseases into the not OIE-listed diseases due to their importance for wild animals and also for early warning purposes, in order to protect human and livestock health. Among these are infection with Crocodilepox virus (Papillomatosis in crocodiles), infection with fibropapillomatosis in sea turtles (Herpesvirus), and infection with Trichinella nelsonei, Zimbabwei and Papouae. Besides of this, released animals interact with local biocenosis, exchange pathogens with other animals and the consequences of such interaction could be unpredictable. Many aspects of ecology and circulation of pathogens are till now not clear.

The one the most traded species among reptiles red-eared slider *(T. scripta)* is world's worst invasive species. And since 1997 the EU has banned on imports of T. scripta elegans. Since 2004, this ban extended to Poland. In Ukraine inexpensive red-eared turtles can be purchased easily not only in pet stores and bird market, but even in the subway and in the souvenir shop. But this pets release by their owners in a nearest pound often. The red-eared slider is very unpretentious and can hibernate even in freezing water easily. A lot of verification of this are in Ukraine and neighboring countries, such as Russia, Litva and Romania. The population of free-living adult red-eared turtles have been detected in Zaporizska and Transcarpathian regions and indicate the urgency of this problem also for the fauna of Ukraine.

Reptile viral studies are limited due to the lack of continuous cell lines in the world cell collections and absolutely absent this cell lines in our country. The aim of our study was to establish a turtle primary cell culture for virological investigation. In first time in Ukraine we describe a new permanent fibroblast-type cell line obtained from disaggregated *T. scripta* internal parenchymal organs pool. To date, the primary culture cells have been successfully subcultured for more than 85 passages and establishment cell culture TF have been obtained. Cell line was optimally maintained at 29-30° C in DMEM and RPMI 1640 medium composition, supplemented with 12% fetal bovine serum. Its karyotype was genetically stable up to 65 passage with a chromosome number of 2n 50. When tested for their sensitivity to several warm-blooded animal viruses, the cell line was

susceptible to a vesicular stomatitis virus (Rabdoviridae), an Aujeszky's disease virus (Herpesviridae) and Newcastle disease virus (Paramyxoviridae) with development of CPE.

Warwick, C. Zoonoses: drawing the battle lines. Veterinary Times Clinical 36 26-28 (2006).
Jacobson, E. R. The desert tortoise and upper respiratory trace disease. Prepared for the Desert Tortoise Preserve Committee, Inc., and U.S. Bureau of Land Management (1992).
Warwick, C. Health and Welfare of Captive Reptiles, (eds Warwick, C. et al.) (Chapman & Hall/Kluwer, London and New York, 2004).
Brown, C. Emerging zoonoses and pathogens of public health significance: an overview. Rev. Sci. Tech. Off. Int. Epiz. 23, 435-442 (2004).
Bringsoe H. NOBANIS Invasive Alien Species Fact Sheet – Trachemys scripta//Online Database of the North European and Baltic Network on Invasive Alien Species – NOBANIS. 2006. //(http://www.nobanis.org)

# PARTIAL CHARACTERIZATION OF A NOVEL ORBIVIRUS ISOLATED FROM CORMORANTS IN ODESSA REGION, UKRAINE

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The genus *Orbivirus* (subfamily *Sedoreovirinae*, family *Reoviridae*) is composed of non-enveloped viruses whose genome consists of 10 segments of linear dsRNA. Virions have icosahedral symmetry, are spherical in form and about 80 nm in diameter. The capsid is organized as three concentric layers of proteins, which surround genome RNA coding for 12 proteins – 8 viral and 4 nonstructural. In the type species – *Bluetongue virus* (BTV), total genome size is 19,200 bp and segments size ranges from 822 to 3,954 bp [1].

International committee on taxonomy of viruses approved 22 species within the genus *Orbivirus* and presented a list of 10 "tentative" orbiviral species [1]. The number of new orbiviruses continues to grow –

based on recent research, at least more 10 isolates were identified as novel orbiviruses [2-5].

Orbiviruses are globally distributed arboviruses. They infect a wide array of vertebrates via a bite of haematophagous arthropods. From 42 approved or proposal orbiviruses species more than half (25) are transmitted by *Culicidae* mosquitoes, the others – by *Ixodida* ticks, *Culicoides* midges or *Phlebotominae* sandflies [1-4].

The most studied are four *Culicoides*-borne orbiviruses – *African horse sickness virus* (AHSV), BTV, *Equine encephalosis virus* (EEV), and *Epizootic hemorrhagic disease virus* (EHDV), that cause severe diseases in domestic and wild ruminants resulting in significant economical losses [6].

To date medical importance of orbiviruses is limited by data on the isolated cases of diseases associated with *Great Island virus* (GIV) – *Kemerovo* (KEMV), *Lipovnik* (LIPV) and *Tribec* (TRBV) serotypes of *Kemerovo antigenic complex*, in the former Czechoslovakia and Russia [7], *Orungo* (ORUV) and *Lebombo* (LEBV) *viruses* – in Nigeria [8], and *Changuinola virus* (CGLV) – in Panama [9].

According to data of the World Organization for Animal Health (OIE) veterinary important orbiviruses have never been reported in Ukraine [10]. To date the only *Kemerovo antigenic complex viruses* of GIV species were revealed in Ukraine. Two strains of TRBV were isolated from *Ixodes ricinus* ticks in Odessa region in 1988 and 2008 [11]. Enzootic territories with *Tribec-Kemerovo viruses* were defined in Zakarpats'ka, Zhytomyrs'ka, and Odessa regions [12].

In this report a partial characterization of a novel orbivirus was presented. The virus was isolated during an arbovirus surveillance program which included ticks, mosquitoes, birds, and small mammalians examination. The strain was recovered from pooled brains of 3 cormorants (*Phalacrocorax spp.*) shot dead in Bilhorod-Dnistrovs'kyi district of Odessa region in June 1990.

Virus isolation and propagation were performed using an intracerebral inoculation of newborn white mice with 10% brain suspension [13]. The disease was developed on day 5 after initial inoculation and characterized as fatal encephalitis. During further passaging the incubation period was reduced to 3-4 days. The virus also caused fatal disease in adult mice after intraperitoneal and subcutaneous inoculation at days 5 and 9, respectively.

Primarily for virus identification transmission electron microscopy and serological assay – complement fixation test (CFT), were used as described earlier [12]. Virions were approximately 70 nm in diameter with a defined surface structure containing ring-shaped capsomeres that is consistent with morphology of viruses of the genus *Orbivirus* within the

family *Reoviridae*. However, CFT of the studied strain antigen with immune sera against CGLV, *Chenuda* (CNUV), *Eubenangee* (EUBV), GIV, *Palyam* (PALV), and *Wad Medani* (WMV) viruses did not reveal any serology reactivity. So, the strain was named *Dnistrovs'kyi orbivirus 408*.

Development of molecular-genetic technology and increasing the amount of data about orbiviral genomes resulted in designing a rapid molecular strategy for orbiviruses detection and characterization [14] that was used for the further identification of *Dnistrovs'kyi orbivirus 408*.

PCR-products were obtained in 3 from 4 designed reaction mixes with *Orbivirus* VP1 consensus PCR forward primers OrbiVP1-F2494-1, OrbiVP1-F2494-2, OrbiVP1-F2494-3 and the reverse primer OrbiVP1-R2682. As a result of the PCR-products genetic analyses VP1 (Pol) protein gene fragment of 134 nucleotides in length was decoded (coincided with the nucleotide positions 2288-2410 for VP1 (Pol) protein gene of CORV strain AUS1960/01, KC853042).

Phylogenetic analysis was conducted with MEGA6 software [15] comparing data of deduced VP1 (Pol) protein fragments of 44 amino acids in length (coincided with the amino acid positions 757-800 for VP1 (Pol) protein of CORV strain AUS1960/01, AGT51054) of *Dnistrovs'kyi orbivirus 408* and 48 known approved or tentative orbiviruses species or strains: AHSV (AJT46700), *Arakonam virus* (AIV43192), BTV (AKP24170, AKV60475), CGLV (AIV43188, ADP88633), CNUV (AHW57716), *Chobar Gorge virus*, CGV (YP_009158901), *Corriparta virus*, CORV (AGT51054, ADP88637, ADP88689, ADP88636, ADP88692, ADP88635, ADP88634, ADP88693), EHDV (ADP88638), EEV (ADU57378), EUBV (ADP88653, AFH41499, AFH41519), *Fenqkai orbivirus* (AKV89254), GIV (AHM93472, AHL27158, AGG68141), *Heramatsu virus*, HERMV (AGZ62525), *Itupiranga virus*, ITUV (ADP88657), *Kasba virus* (AIV43191), LEBV (AFX73376), *Matucare virus*, MATV (ADP88658), *Megami Mountain virus* (BAT21343), *Mobuck virus* (AGX89720), ORUV (ADP88659), PALV (ADP88667), *Peruvian horse sickness virus*, PHSV (ADP88670), *Sathuvachari virus*, SVIV (AGE32260, ADP88690), *Tibet virus*, TIBOV (AHE77361), *St. Croix River virus*, SCRV (AAG34363), *Umatilla virus*, UMAV (ADP88672, ADP88694), WMV (AHX25713), *Wallal virus*, WALV (ADP88677, AIT55723), *Warrego virus*, WARV (ADP88680), *Wongorr virus*, WGRV (ADP88682), *Yunnan virus*, YUOV (AAW28767, ADP88688).

Phylogenetic analyses revealed the *Dnistrovs'kyi orbivirus 408* strain was the most closely related to mosquito-borne CORV. CORV has been detected in Australia, Africa and South America. CORV strains have been isolated from wild birds, and neutralizing antibodies were found in wild and domestic birds, cattle, marsupials, horses and man [16].

Amino acid sequence of VP1 (Pol) protein fragment of *Dnistrovs'kyi orbivirus 408* was different from those of CORV strains by the presence of 2 or 3 amino acid substitutions (levels of identity were 95,45% or 93,18%, respectively). *Dnistrovs'kyi orbivirus 408* had histidine (H) at the position 765 and alanine (A) at the position 767 while all studied CORV strains had tyrosine (Y) and serine (S), respectively. Additional differences were detected by comparison *Dnistrovs'kyi orbivirus 408* with the CORV strain DPP3309 (ADP88636) that had arginine (R) instead cysteine (C) at the position 795 and with the CORV strain DPP3297 (ADP88637) that had phenylalanine (F) instead serine (S) at the position 799.

Levels of identity of *Dnistrovs'kyi orbivirus 408* with other orbiviruses, except for CORV, were significantly lower and varied from 43,18% to 56,82% (the number of amino acid differences was ranged from 19 to 25).

In summary, the novel virus named *Dnistrovs'kyi orbivirus 408* – a member of genus *Orbivirus* (family *Reoviridae*) close related to mosquito-borne CORV, was isolated from birds in southern Ukraine. These data suggest that in addition to the tick-borne GIV (TRBV serotype) another orbiviral species infected birds *Phalacrocorax spp.* and transmitted, probably, by mosquitoes occurred in southern Ukraine. For more precise determination of the taxonomic position of the *Dnistrovs'kyi orbivirus 408* the phylogenetic analysis of the whole-genome sequence data is necessary. Isolation of *Dnistrovs'kyi orbivirus 408*-like virus from vectors would enable to define its ecological links.

1. Virus Taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses / Ed. A.M.Q. King, M.J. Adams, E.B. Carstens, E.J. Lefkowitz. – Elsevier, 2012. – 1326 p. – ISBN: 978-0-12-384684-6.

2. Martins L.C. Characterization of Minacu virus (Reoviridae: Orbivirus) and pathological changes in experimentally infected newborn mice / L.C. Martins, J.A.P. Diniz, Silva E.V.P. [et al.] // Int. J. Exp. Path. – 2007. – Vol. 88. – P. 63–73.

3. Firth C. Detection of Zoonotic Pathogens and Characterization of Novel Viruses Carried by Commensal Rattus norvegicus in New York City / C. Firth, M. Bhat, M.A. Firth [et al.] // MBio. – 2014. – Vol. 5, № 5. – e01933-14.

4. Belaganahalli M.N. Genetic characterization of the tick-borne orbiviruses / M.N. Belaganahalli, S. Maan, N.S. Maan [et al.] // Viruses. – 2015. – Vol. 7, № 5. – P. 2185-2209.

5. Ejiri H. Genetic and biological characterization of Muko virus, a new distinct member of the species Great Island virus (genus Orbivirus,

family Reoviridae), isolated from ixodid ticks in Japan / H. Ejiri, C.K. Lim, H. Isawa [et al.] // Arch. Virol. – 2015. – Vol. 160, № 12. – P. 2965-2977.

6. MacLachlan N.J. Re-emergence of bluetongue, African horse sickness, and other Orbivirus diseases / N.J. MacLachlan, A.J. Guthrie // Vet. Res. – 2010. – 41:35. – DOI: 10.1051/vetres/2010007.

7. Libikova H. Orbiviruses of the Kemerovo complex and neurological diseases / H. Libikova, F. Heinz, D. Ujhazyova, D. Stunzner // Med. Microbiol. Immunol. – 1978. – Vol. 166, № 1-4. – P. 255-263.

8. Moore D.L. Arthropod-borne viral infections of man in Nigeria, 1964-1970 / D.L. Moore, O.R. Causey, D.E. Carey [et al.] // Ann. Trop. Med. Parasitol. – 1975. – Vol. 69, № 1. – P. 49-64.

9. Silva S.P. Genetic and biological characterization of selected Changuinola viruses (Reoviridae, Orbivirus) from Brazil / S.P. Silva, M. Dilcher, F. Weber [et al.] // J. Gen. Virol. – 2014. – Vol. 95. – P. 2251-2259.

10. World Animal Health Information Database (WAHID Interface) / World Organization for Animal Health (OIE) // Available at: http://www.oie.int/wahis/public.php?page=home.

11. Dedkov V.G. Characterization of Two Strains of Tribec Virus Isolated in Ukraine / V.G. Dedkov, D.A. Dubina, O.A. Yurchenko [et al.] // Vector-Borne and Zoonotic Diseases. – 2014. – Vol. 14, № 11. – P. 808-816.

12. Enzootic areas with especially dangerous natural foci infections in Ukraine and measures of their prevention. Information letter / SI “Central Sanitary-Epidemiological Station of the MoH of Ukraine”, L’viv Research Institute for Epidemiology and Hygiene. – Kyiv, 2011. – 138 p.

13. Gould E.A. Growth, titration and purification of alphaviruses and flaviviruses / E.A. Gould, J.C.S. Clegg // Virology - a practical approach; ed. B.W.J. Mahy. – Oxford: IRL, 1985. – P. 43–78.

14. Palacios G. Rapid Molecular Strategy for Orbivirus Detection and Characterization / G. Palacios, C. Cowled, A.V. Bussetti [et al.] // J. Clin. Microbiol. – 2011. – Vol. 49, № 6. – P. 2314-2317.

15. Tamura K. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0 / K. Tamura, G., Stecher D. Peterson [et al.] // Molecular Biology and Evolution. – 2013. – Vol. 30. – P. 2725-2729.

16. Belaganahalli M.N. Full Genome Sequencing of Corriparta Virus, Identifies California Mosquito Pool Virus as a Member of the Corriparta virus Species / M.N. Belaganahalli, S. Maan, N.S. Maan [et al.] // PLoS ONE. – 2013. – Vol. 8, № 8. – e70779. – DOI:10.1371/journal.pone.0070779.

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# ABSTRACTS

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