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MOLECULAR AND GENETIC CHARACTERISTICS ISOLATES *VIRUS OF PORCINE ENZOOTIC ENCEPHALOMYELITIS* ISOLATED FROM WILD BOAR AND DOMESTIC SWINE ON THE TERRITORY OF UKRAINE

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The article provide data about detection two isolates viruses of porcine enzootic encephalomyelitis from the territory of Ukraine isolated from domestic piglet and wild boar. Porcine enzootic encephalomyelitis virus isolate «Shevchenkovo-2005» was collected from domestic piglet in 2005 in private housing on the territory of Kyiv region. The virus isolate «Novoazovsky-2013» was detected from shot wild boar on the territory of Lugansk region. The initial was done the indication of the viruses in cell culture of pig kidney (PK) was performed with their further identification for sensitivity to chloroform. PCR technique was used for detecting of species-specific RNA virus of porcine enzootic encephalomyelitis with subsequent VP1 cDNA sequencing. The results of estimations showed that above-mentioned samples belong to PTV 1 Teschovirus group. The designed by us primers that would be specific for almost all serotypes of porcine Teschoviruses it was analyzed by nucleotide sequences encoding VP1 of 11 PTV available serotypes. This allowed us for detection of the conservative region and designing of the oligonucleotide primers (PTV-F, PTV-R). Phylogenic analysis of amino acid sequences of capsid protein VP1 of Teschoviruses from Ukrainian isolates and different serotypes have shown that the viruses from Ukraine belong to the first Teschovirus group (PTV1). There were found some point mutations in the isolates may be due to high mutational rate as they are single-strained RNA viruses and its observation is peculiar for all members of Picornaviridae family.

Keywords: TESCHEN DISEASE VIRUSES, DOMESTIC PIGLET, WILD BOAR, IDENTIFICATION, POLYMERASE CHAIN REACTION, SEQUENCING, PHYLOGENIC ANALYSIS

МОЛЕКУЛЯРНО-ГЕНЕТИЧНА ХАРАКТЕРИСТИКА ІЗОЛЯТІВ ВІРУСУ ЕНЗООТИЧНОГО ЕНЦЕФАЛОМІЄЛІТУ СВИНЕЙ ВИДІЛЕНИХ ВІД ДИКОГО КАБАНА І ДОМАШНЬОГО ПІДСВИНКА НА ТЕРИТОРІЇ УКРАЇНИ

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У статті приведені результати виявлення двох ізолятів вірусу ензоотичного енцефаломієліту свиней з території України: від домашнього підсвинка і дикого кабана. Ізолят вірусу ензоотичного енцефаломієліту свиней «Шевченково-2005» був виділений від хворого підсвинка у 2005 році у приватному секторі на території Київської області. Ізолят вірусу ензоотичного енцефаломієліту свиней «Новоазовський-2013» був виділений від відстріляного дикого кабана з

території Луганської області. Спершу було проведено індикацію ізолятів вірусу у культурі клітин СНЕВ з їх подальшою ідентифікацією за чутливості до хлороформу. Для виявлення РНК вірусу ензоотичного енцефаломієліту свиней було використано полімеразну ланцюгову реакцію з наступним секвенуванням VP1 кДНК. Результати дослідження показали, що згадані вище ізоляти відносяться — до першої групи Teschovirus — PTV 1. Розробленими нами праймерами, які були б специфічні для майже всіх серотипів Teschoviruses, проаналізовані нуклеотидні послідовності, що кодують VP 1 із 11 доступних серотипів. Це дозволило виявити консервативний регіон за допомогою дизайну олігонуклеотидных праймерів (PTV-F, PTV-R). Філогенетичний аналіз амінокислотних послідовностей капсидного білка VP 1 Teschoviruses українських ізолятів і різних серотипів показали, що віруси України відносяться до перщої Teschovirus групи (PTV 1). Виявлені деякі точкові мутації ізолятів можуть бути пов'язані з їх рекомбинацією, що властиво для всіх членів родини Picornaviridae. Виявлені послідовності українських ізолятів були занесені в GenBank (KJ081865, KJ081866 та KJ081867).

Ключові слова: ВІРУС ХВОРОБИ ТЕШЕНА, ДОМАШНІЙ ПІДСВИНОК, ДИКИЙ КАБАН, ІДЕНТИФІКАЦІЯ, ПОЛІМЕРАЗНА ЛАНЦЮГОВА РЕКЦІЯ, СЕКВЕНУВАННЯ, ФІЛОГЕНЕТИЧНИЙ АНАЛІЗ

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ИЗОЛЯТОВ ВИРУСА ЭНЗООТИЧЕСКОГО ЭНЦЕФАЛОМИЕЛИТА СВИНЕЙ, ВЫДЕЛЕННЫХ ОТ ДИКОГО КАБАНА И ДОМАШНЕГО ПОДСВИНКА НА ТЕРРИТОРИИ УКРАИНЫ

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В статье приводятся результаты обнаружения двух изолятов вируса энзоотического энцефаломиелита свиней с территории Украины: от домашнего подсвинка и дикого кабана. Изолят вируса энзоотического энцефаломиелита свиней «Шевченково-2005» был выделен от больного подсвинка в 2005 году в частном секторе на территории Киевской области. Изолят вируса энзоотического энцефаломиелита свиней «Новоазовский-2013» был выделен от отстрелянного дикого кабана на территории Луганской области. Сначала была проведена индикация изолятов вируса в культуре клеток СПЕВ с их дальнейшей идентификацией по чувствительности к хлороформу. Для обнаружения РНК вируса энзоотического энцефаломиелита свиней была использована полимеразная цепная реакция с последующим секвенированием VP1 кДНК. Результаты исследования показали, что упомянутые выше изоляты относятся — к первой группе Teschovirus — PTV 1. Разработаными нами праймерами, которые были бы специфичны для почти всех серотипов Teschoviruses, проанализированы нуклеотидные последовательности, кодирующие VP 1 из 11 доступных серотипов. Это позволило обнаружить консервативный регион с помощью дизайна олигонуклеотидных праймеров (PTV-F, PTV-R). Филогенетический анализ аминокислотных последовательностей капсидного белка VP 1 Teschoviruses украинских изолятов и различных серотипов показали, что вирусы Украины относятся к первой Teschovirus группе (PTV 1). Выявленные некоторые точечные мутации изолятов могут быть связаны с их рекомбинацией, что свойственно для всех членов семейства Picornaviridae. Выявленные последовательности украинских изолятов были занесены в GenBank (KJ081865, KJ081866 та KJ081867).

Ключевые слова: ВИРУС БОЛЕЗНИ ТЕШЕНА, ДОМАШНИЙ ПОДСВИНОК, ДИКИЙ КАБАН, ИДЕНТИФИКАЦИЯ, ПОЛИМЕРАЗНАЯ ЦЕПНАЯ РЕКЦИЯ, СЕКВЕНИРОВАНИЕ, ФИЛОГЕНЕТИЧЕСКИЙ АНАЛИЗ

Porcine enzootic encephalomyelitis (Teschen disease) is a viral disease characterized by encephalomyelitis and paralyses [1]. At first, Trephni registered enzootic disease in 1930 in the of Teschen town (Czech Republic) [1, 2]. Since that time, the disease was registered in Europe, North America, South America, Africa, Asia [3, 4], Australia [2]. RNA virus is the agent for this disease. The species of the virus *encephalomyelitis* belongs to the *Picornaviridae* family, *Enterovirus* genus [5, 6], that represent the first serotype of pig's *Enteroviruses* [4, 7]. Based on studies and data accumulation on virus genetic structure, taxonomy and nomenclature of *Picornaviruses* were changed [8] at the 11th International Congress on Virology in 1999 in Sidney. *Enterovirus* genus, in particular, pig *Enteroviruses* were changed. Based on study findings [9–11] International Committee on Taxonomy reclassified pig *Enteroviruses* into three types that belong to two genuses. Pig's *Enteroviruses* of serotype 1–7, 11–13 and one new serotype [10] classified as *Porcine Teschoviruses* (PTV). The last one contains 11 serotypes and classified as a specific *Teschoviruses*. Pig's *Enteroviruses* of the 8th serotype reclassified as *Porcine Enterovirus* A (PEV-A), 9 and 10 as *Porcine Enterovirus* B (PEV-B) and belongs to *Enterovirus* genus. According to Y. Kaku [12] it was determined that RNA of PTV includes IRES elements of the fourth classes of *Picornavirus*.

Laboratory diagnostics of Porcine enzootic encephalomyelitis is performed by the following techniques as complement fixation test, enzyme-linked immunosorbent assay (ELISA), neutralization test [2, 7, 13, 14] and fluorescent antibody test [2, 7, 14]. The World Organization of Animal Health (OIE) recommends the specific laboratory techniques to identify antigen of Teschen disease virus as neutralization test in sensitive culture cells, fluorescent antibody test, ELISA and PCR; for serology the neutralization test and ELISA [4, 7].

Currently, molecular and genetic evaluation of pig *Enteroviruses* strains is the main issue because determination of sequence in different gene sites allowing to obtain characteristics of viruses molecular peculiarities. In Ukraine, the researchers [15] developed specific primers for molecular and genetic identification of *Teschoviruses* and pig *Enteroviruses* and showed their specificity. Using this sequence, the scientists from China determined close relations between isolated viruses and Talfan strain by homology of amino acids and nucleotides of 98.9 % and 99.5 %, respectively [16]. During the outbreak of Teschen disease among local pigs on Haiti Island [17], the scientists isolated samples of the virus from the site and performed their sequencing. The sequencse identity between the Haitian sample and other 16 PTV strains were at 80–86 % and 69–83 % for the entire genome and the VP1 region, respectively. The results of phylogenetic analysis of strain PTV polyprotein showed that Haiti virus closely related with PTV strains of the first serotype (PTV-1). Porcine enzootic encephalomyelitis virus isolated on the territory of Canada was at 99 % similar to PTV serotype 1 (Talfan strain) and 97 % similar to Konratice strain. Nucleotide sequence was at 96 % similar to Talfan strain and 97 % to Konratice straine [18].

There was information on the sensitivity of wild pigs to porcine enzootic encephalomyelitis virus on the territory of Europe and river pigs on Madagascar [2, 7]. However, the scientists did not study that issue enough due to the complexity of monitoring studies among the population of such wild animals.

Two samples of porcine enzootic encephalomyelitis virus (one from domestic pig — 2005 and another — from wild boar 2012) were isolated and identified in the Laboratory of Pig Diseases and Biotechnology the Institute of Veterinary Medicine of NAAS, Ukraine. This study was curried out to isolate field wild-type and domestic porcine enzootic encephalomyelitis virus circulating in Ukraine and obtain molecular sequence information about the genome region encoding viral protein 1 (VP1) to explore the peculiarities among this distinct isolates and provide new

approach for monitoring and surveillance under *Teschovirus* infection.

Materials and methods

Porcine enzootic encephalomyelitis virus isolate № 1 «Novoazovsky-2013» was indicated from the 10-month-old wild boar (rectal swabs) in Novoazovsk district of Donetsk region in 2013. Infectious activity titer of the seventh recultivation passage of virus was 6,25 lg $TCID_{50}/cm^3$ in cell culture PK.

Porcine enzootic encephalomyelitis virus isolate № 2 «Shevchenkovo-05» was indicated from the 3 month-old domestic piglet (brain) in Brovary district of Kyiv region in 2005. Infectious activity titer in the seventh recultivation passage of virus was 7,29 lg $TCID_{50}/cm^3$ in cell culture PK.

Amplification and sequencing of VP1 region of porcine enzootic encephalomyelitis virus isolates:

RNA extraction. Viral RNA was extracted from 100 µl of the virus containing fluid after the seventh recultivation of each sample using Ribosorb reagent kit (Amplisense, Russia), according to the manufacture's instruction. Briefly, 100 µl of virus containing fluid was added to 300 µl of Lysis reagent and incubated at room temperature during 10 min., after incubation 20 µl of silica resin suspension was added to lysed samples and incubated during 10 min. at room temperature. Samples were centrifuged 30 s. at high speed (13,000 rpm) using microcentrifuge MiniSpin® (Eppendorf). Pellets of silica resin were washed twice using Washing buffer and dried at room temperature. RNA was eluted from silica resin in a total volume of 60 µl DEPC-treated water and stored frozen at 80 °C.

RT-PCR amplification cloning and sequencing. Viral RNA was reverse transcribed using Reverta-L reagent kit (Amplisense, Russia), according to the manufacture's instruction, briefly 5 µl of viral RNA was incubated 40 min at 37 °C with 50 ng of random hexamers and 200 U of M-MLV Reverse Transcriptase in supplied RT buffer. Finally, 5µl of cDNA was amplified in 25 µl of reaction mixture contained 50.0 mM Tris-HCl (pH 8.3), 50.0 mM KCl, 3.0 mM $MgCl_2$, 10.0 mM DTT, 0.4 mM each of dNTP, 0.5 U Pfu DNA polymerase (Thermo Scientific, Lithuania) and 5.0 pM of degenerated oligonucleotide primers pair: PTV-F (5'-CAGCCRGCWGAGACAGG-3') and PTV-R (5'-GTRAATGARGGYAATGC-3'). Amplification conditions involved an initial denaturing step of 94 °C for 2 min, followed by 35–40 cycles each comprising denaturation at 94 °C for 15 sec, annealing at 57 °C for 15 sec and extension at 72 °C for 30 sec. The resulting amplified cDNAs were visualized by electrophoresis in 1.5 % agarose gel stained with ethidium bromide and analyzed in UV rays using FisherBiotech™ Ultraviolet Transilluminator. The amplified 427 bp fragments, which corresponded in location to nucleotide 2476 to 2903 of the Porcine teschovirus 1 (PTV-1) genomic sequence (GenBank accession number AB038528), was excised from agarose gel, column purified using GeneJET PCR Purification Kit (Thermo Scientific, Lithuania) according manufacture manual. Purified PCR products were cloned using CloneJet PCR Cloning Kit (Thermo Scientific, Lithuania), briefly 1 µl of purified PCR product was incubated with 1 µl of DNA blunting enzyme in 18 µl of reaction mixture at 70 °C for 5 min and chilled on ice, after that in reaction mixture were added 1 µl of pJET1.2/blunt Cloning Vector (50 ng) and 1 µl of T4 DNA Ligase (5U). Reaction mixture was incubated 5 min. at room temperature and directly transformed into C-Max™ 5α *E. coli* competent cells (Bio-Rad) using heat shock (42 °C) procedure. Transformed *E. coli* cells were plated on LB-agar plate supplemented with 100 mkg of Ampicillin and incubated overnight at 37 °C. Recombinant *E. coli* colonies were picked into 3 ml LB medium with 100 mkg Ampicillinum and growing overnight with shacking at 37 °C in bacterial incubator. Recombinant plasmid DNA was purified from overnight culture of *E. coli* using QIAprep Spin Miniprep Kit (Qiagen), according manufacture manuals. At least 2 representative recombinant plasmids, each containing one of the 427 bp PTV-specific inserts, were sequenced using pJET1.2 forward and reverse primers. Sequencing analysis was performed using Genetic

Analyzer ABI 3130 (Applied Biosystems, USA) and Reagent Kit «BigDye®terminator, v.3.1» due to manufacturer's manual. Sequencing datas were analyzed and edited by the Chromas Lite2.1.1. software (Technelysium, Australia).

Sequencing analysis. The consensus sequences obtained were compared to nucleotide sequences in GenBank database (NCBI) using BLAST search tool. Derived nucleotide sequences were aligned by using on-line Multalign tool (http://multalin.toulouse.inra.fr), predicted amino acid translation sequence were aligned by using on-line Praline tool (http://zeus.few.vu.nl). Phylogenic analysis amino acid sequence of viral capsid VP1 of different *Techovirus* serotypes was performed by using on-line tool ClustalW2 http://www.ebi.ac.uk/Tools/phylogeny/ clustalw2 _phylogeny/). As a result the consensus tree was calculated.

Results and discussion

Diagnosis and characterization of pathogenic properties of field wild-type and domestic porcine enzootic encephalomyelitis virus isolates.

In previous studies we found that after 7 successive passages in SKES cell culture line increased an infective action of virus, isolated from rectal washouts up to 6,25±0,09 lg $TCID_{50}/cm^3$, from brain tissues up to 7,29±0,07 lg $TCID_{50}/cm^3$.

Further identification of the porcine enzootic encephalomyelitis virus in neutralization test with antigenic relationship detection of the selected isolates to the strain of Teschen disease «Perechynsky 642» The titer of the virus strain porcine enzootic encephalomyelitis «Perechynsky 642» before the reaction was 8,73±0,13 lg $TCID_{50}/cm^3$. The infectious activity of isolates 7th passage were in such position as from rectal swabs 6,25±0,09 lg $TCID_{50}/cm^3$ and from the brain 6,29±0,07 lg $TCID_{50}/cm^3$. Specific serum titer of strain «Perechynsky 642» was 1:1000, and it was used 20 doses of neutralizing serum for this test.

The results of neutralization test with constant serum dose showed that this isolates belong to porcine enzootic encephalomyelitis virus [19].

Amplification and sequencing of VP1

Prior to design oligonucleotide primers which span almost all serotypes of porcine enzootic encephalomyelitis virus we have analyzed nucleotide sequences encoding VP1 of about 11 PTV serotypes available. This allowed us to search conservative region and design degenerate oligonucleotide primers (PTV-F, PTV-R).

Specific PCR product near 427 bp was amplified from cDNA of the sample 1 (isolate from wild boar) and 2 (isolate from domestic piglet) of porcine enzootic encephalomyelitis virus (Fig. 1).

During sequence analysis of several recombinant clones we have found that VP1 gene of *Teschoviruses*, sample № 1 was monomorphic, while sample № 2 had at least two forms (2a, 2b) (Fig. 2)

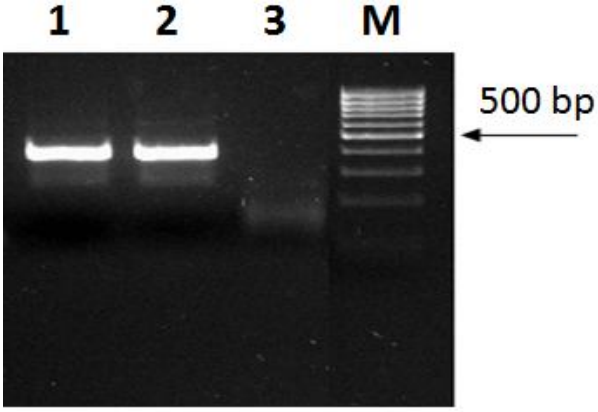

Fig. 1. Electrophoregram of PCR products separated in 1.5 % agarose gel and stained by ethidium bromide in UV rays. 1 — cDNA sample 1; 2 — cDNA sample 2; 3 — negative control (water); M — marker of DNA fragment length (SM1143, Fermentas). The arrow marks a fragment of 500 bp

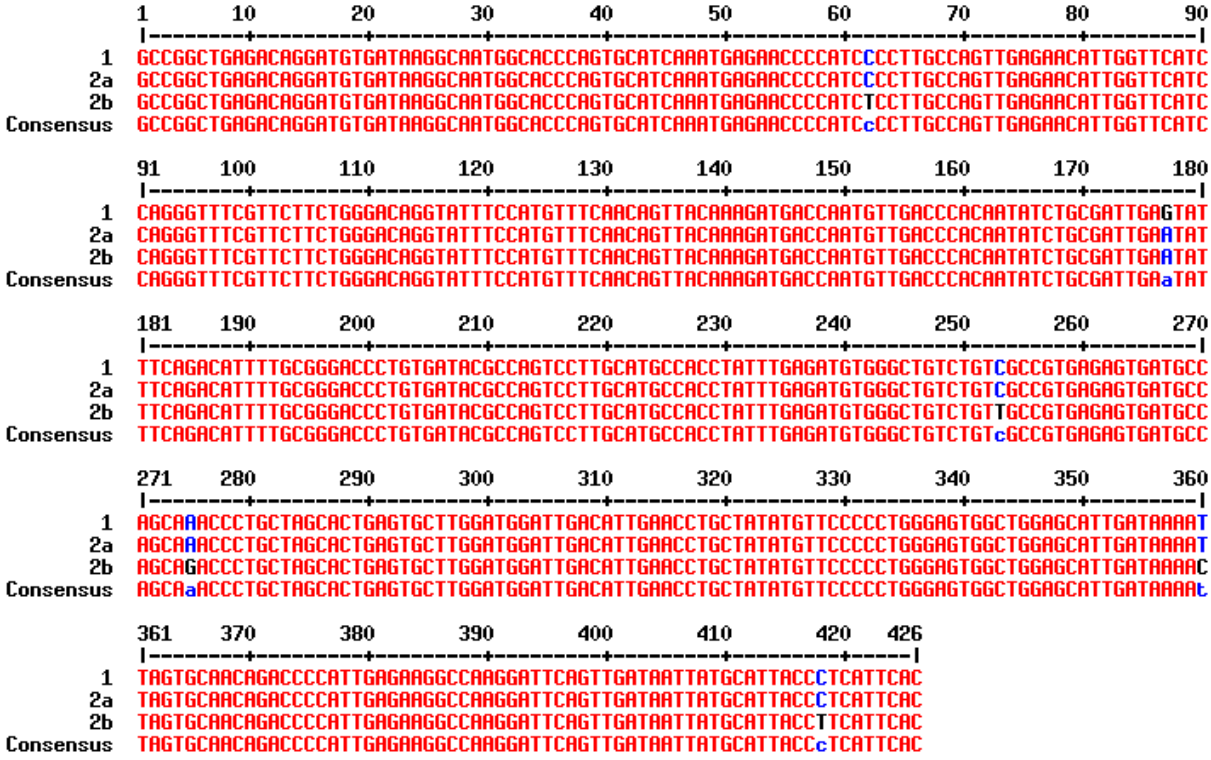

Fig. 2. VP1 gene alignments of two Teschovirus isolates by using web tool Multalign (http://multalin.toulouse.inra.fr)

Figure 2 shows observed polymorphism VP1 gene *Teschoviruses* of both estimated isolates. Analyzing polymorphic sites within VP1 gene, we can see the following patterns, that found difference between isolates №1 and № 2 is only in single nucleotide replacement $G_{176}A$. Other five replacements $C_{62}T$, $C_{253}T$, $A_{274}G$, $T_{360}C$ та $C_{417}T$ are specific only for 2a and 2b clones within virus isolate № 2.

Table describes detected nucleotide replacements and amino acids coded by changed triplets. It was set, that two replacements $C_{253}T$ and $C_{417}T$ are specific for clone 2a and there are nonsense mutations that do not change amino acids V (Valine). Other three nucleotide replacements specific for clone 2b are $C_{62}T$, $A_{274}G$ and $T_{360}C$ causes amino acid changes — $P_{21}S$, $D_{92}N$ and $I_{120}T$.

Table

Specific polymorphism within capsid protein (VP1) of Ukrainian isolates of porcine enzootic encephalomyelitis virus

Nucleotide replacement	Amino acid replacement	Isolate 1	Isolate 2	
			Clone 2a	Clone 2b
$C_{62}T$	$P_{21}S$	P	P	S
$G_{176}A$	$N_{59}S$	S	N	N
$C_{253}T$	V_{84} (nonsense)	V	V	V
$A_{274}G$	$D_{92}N$	N	N	D
$T_{360}C$	$I_{120}T$	I	I	T
$C_{417}T$	P_{139} (nonsense)	P	P	P

Note: P — Proline; S — Serine; V — Valine; N — Asparagine; I — Isoleucine; D — Aspartic acid; T — Threonine

Based on obtained data, two virus isolates are different only in one replacement $N_{59}S$ on the amino acid level. At that, S_{59} (Serine) is specific only for Ukrainian field virus isolate № 1 comparing to different *Techovirus* serotypes, though amino acid N (Asparagine) is specific for the most serotypes at this site (Fig. 3).

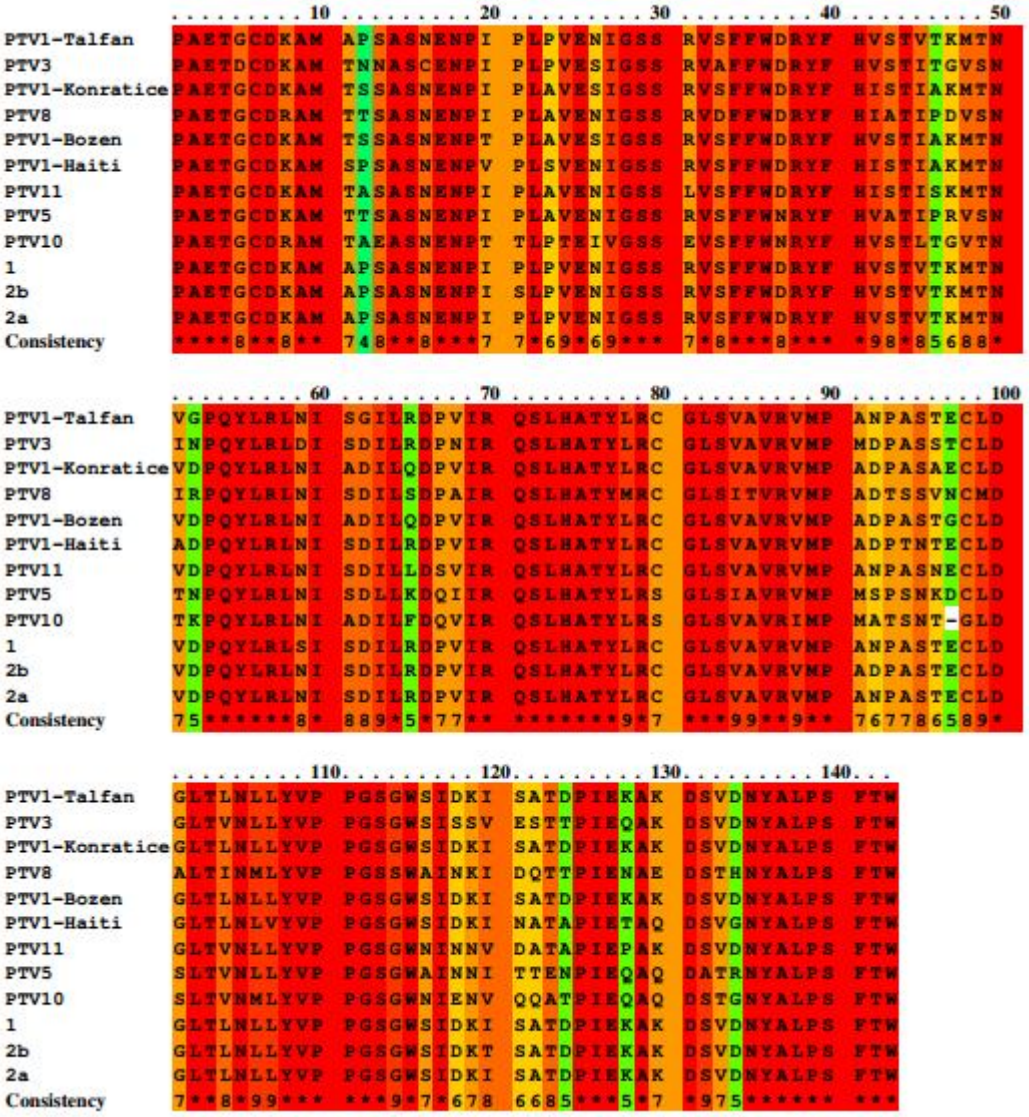

Fig. 3. VP1 aminoacid alignments of different Techoviruses serotypes using on-line tool Praline (http://zeus.few.vu.nl)

Amino acid S_{21} is specific only for virus isolate of clone 2a, though amino acid P (Proline) is specific for all analyzed serotypes at this position. Analysis of amino acid replacement $D_{92}N$ shows that it is specific for different *Teschovirus* serotypes. At this position, PTV3, PTV1-Kontratice, PTV-8, PTV1-Bozen and PTV1-Haiti serotypes contain amino acid D (Aspartate), serotypes PTV1-Talfan and PTV11 code Asparagine (N). Replacement of $I_{120}T$, is only specific for virus isolate № 2 (clone 2b). Amino acids I (Isoleycine) and V (Valine) are present in different serotypes at this position though this site is not conservative for different serotypes of PTV.

Phylogenic analysis of amino acid sequences of capsid protein VP1 of Ukrainian *Teschoviruses* and different serotypes show that the Ukrainian viruses belong to the first *Teschovirus* group (PTV1). Figure 4 presents Phylogenetic tree for individual position of the Ukrainian viruses within the cluster of the first *Teschovirus* group, including PTV1-Haiti, PTV1-Kontratice, PTV1-Bozen and PTV-Talfan, and the virus isolate № 1 occupies intermediate position between virus's clones 2a and 2b.

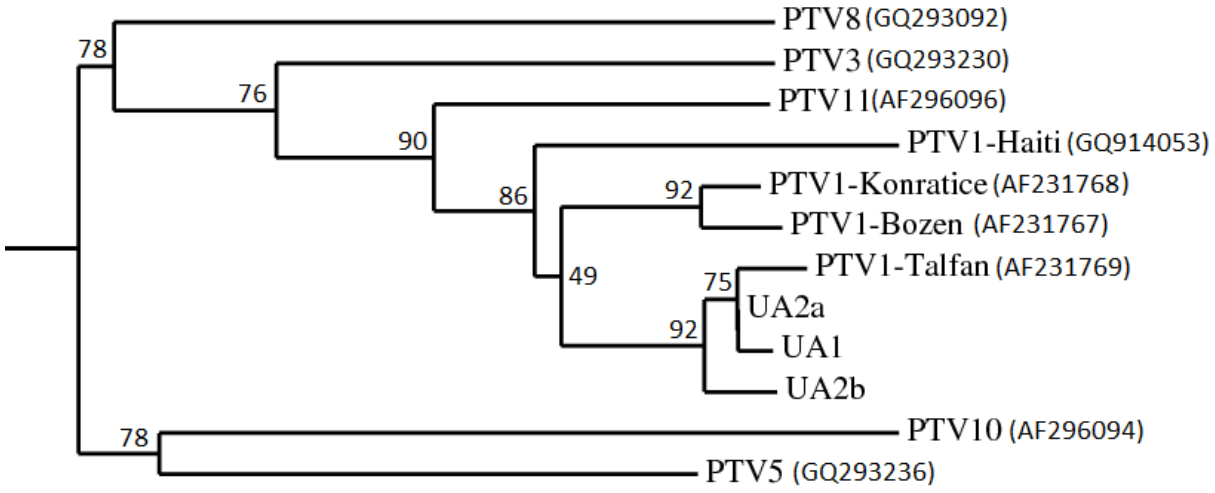

Fig. 4. Phylogenetic tree is based on partial nucleotide sequences of viral capsid VP1 gene of different *Teschovirus* serotypes. Likelihood of cluster separation shows in percent; each serotype has indicated GenBank number. Ukrainian viruses isolates are UA1, UA2a and UA2b respectively

As a result, we can conclude that both isolates of *Teschoviruses*, circulated in Ukraine, field wild-type and domestic one, belong to PTV1 serotype group. Found some point mutation in the isolate 2 may be due high mutational rate as they are single-strained RNA viruses and this observation is common for all members of *Picornaviridae* family.

We may assume that the specific amino acid replacements in sequence of capsid protein VP1 of Ukrainian *Teschoviruses*, such as, $N_{59}S$ in virus isolate 1 and $P_{21}S$ and $I_{120}T$ in virus isolate 2b can influence on antigenic properties of isolated viruses.

It will be very important for future studies on development of new approaches for control and surveillance by porcine enzootic encephalomyelitis virus in Ukraine.

Identified sequences of Ukrainian *Teschoviruses* were deposited in GenBank (KJ081865, KJ081866 та KJ081867).

Nowadays it was counted about 8 million of pigs and near 4.5 millions are in private farm properties. Density of pig population is the highest in the Western part of Ukraine (Ternopil, Zakarpatye, Ivano-Frankivsk and Chernivtsy regions), Cherkasy and Donetsk regions. The lowest density is in the Southern (Republic of Crimea, Kherson and Mykolayv regions). Northern (Zhitomyr, Chernigiv, Sumy regions) and Eastern parts (Kharkiv, Lugansk regions).

The population of wild boars is growing annually and in 2012 it was counted near 65 thousands animals. According to the Fig.2 number of wild boars is higher in the Western regions and Polissya area and less in the in South and East part of the Ukrainian territory.

Above results confirmed the stating what on Ukraine territory detected 2 isolated of porcine enzootic encephalomyelitis virus is one from wild boar and another from domestic pig. Obtained results proves a high level of homology between viruses (isolated from domestic piglet and wild boar from the territory of Ukraine) by sequences on 99 %, amino acid composition on 95% and similar to PTV1 strains. Biological material was taken from different parts of Ukraine proves that porcine enzootic encephalomyelitis virus circulate among the population of wild boars and domestic pig and they are similar to each other. Further studies should be focused on isolation of porcine enzootic encephalomyelitis virus from other regions of Ukraine for detailed analysis.

It is important that in despite of major territory the size of spots where investigated isolates was detected and some different

biological objects they was detected of, it was confirmed the 99 % homology between them. It was confirmed the circulation of the homological isolates of porcine enzootic encephalomyelitis virus among domestic pigs and wild boars onto Ukraine territory.

Conclusions

1. As a result of the studies, two porcine enzootic encephalomyelitis virus isolates were isolated and identified on the territory of Ukraine: «Shevchenkovo-2005» virus isolated from the brain of sick piglet of three-month-old with specific clinical signs (neurological disorders) and virus «Novoazovsky-2013» isolated from the large intestines (rectal swabs) of healthy wild boar.

3. Identification of the virus isolates was determined by using PCR-detection of specific RNA to porcine enzootic encephalomyelitis virus and sequencing of VP1 gene cDNA. The results showed that the viruses were 99 % similar on sequences and 95 % in amino acid composition and their similarity to PTV 1.

4. Both isolates of porcine enzootic encephalomyelitis virus, circulated in Ukraine, the wild-type and domestic one, belong to PTV1 serotype group. Determined specific amino acid replacements in the sequence of VP1 capsid protein in Ukrainian *Teschoviruses*, i.e., $N_{59}S$ in virus №1 and $P_{21}S$ i $I_{120}T$ of the virus № 2b is able to influence on antigenic properties of isolated viruses.

5. Obtained results about the sequence of VP1 gene from isolated viruses have practical significance for future studies on development of new preventive measures against porcine enzootic encephalomyelitis virus in Ukraine.

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