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АНТИВІРУСНИЙ ТА ІМУНОСТИМУЛЮЮЧИЙ ПОТЕНЦІАЛ ФТОРВМІСНИХ ТРІАЗОЛІВ

Проблема пошуку ефективних протівірусних препаратів зумовлена високою захворюваністю і широким розповсюдженням вірусних інфекцій. Метою представленої роботи було дослідити антигерпетичну активність фторованих нуклеозидних сполук G8 та G9 (2-N-заміщені-4-тозил-5-поліфторалкіл-1,2,3-триазолі) на моделі *invivo* та визначити їх імуномодулюючий потенціал. Показано значне інгібування репродукції вірусу під дією досліджуваних сполук в концентраціях 0,4 та 0,5 мг/кг, що було в рази ефективніше дії ацикловіру. Коефіцієнт захисту становив 80%. Встановлено збільшення рівня ІФН γ та ІЛ-2 в сироватці крові, що вказує на наявний імуномодулюючий ефект фторованих нуклеозидних сполук. Проведені дослідження дозволяють стверджувати про наявність антигерпетичної, імуностимулюючої дії фторованих триазолів та необхідність поглибленого вивчення механізмів даного процесу.

Ключові слова: HSV-1, фторовані нуклеозидні сполуки, антигерпетична активність.

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АНТИВИРУСНЫЙ И ИМУНОСТИМУЛИРУЮЩЕЕ ПОТЕНЦИАЛ ФТОРСОДЕРЖАЩИХ ТРІАЗОЛОВ

Проблема поиска эффективных противовирусных препаратов обусловлена высокой заболеваемостью и широким распространением вирусных инфекций. Целью представленной работы было исследовать антигерпетические активности фторированных нуклеозидных соединений G8 и G9 (2-N-замещены-4-тозил-5-полифторалкіл-1,2,3-триазола) на модели *invivo* и определить их имуномодулирующий потенциал. Показано значительное ингибирование репродукции вируса под действием исследуемых соединений в концентрациях 0,4 и 0,5 мг/кг, что было в разы эффективнее действия ацикловира. Коэффициент защиты составлял 80%. Установлено увеличение уровня ИФН γ и ИЛ-2 в сыворотке крови, что указывает на имеющийся имуномодулирующий эффект фторированных нуклеозидных соединений. Проведенные исследования позволяют утверждать о наличии антигерпетического, имуностимулирующего действия фторированных триазолов и необходимости углубленного изучения механизмов данного процесса.

Ключевые слова: HSV-1, фторированные нуклеозидные соединения, антигерпетическая активность.

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GENETIC CHARACTERIZATION OF INFECTIOUS Bursal DISEASE VIRUS ISOLATES IN UKRAINE

The objective of the investigation was to characterize infectious bursal disease viruses (IBDV) circulating in commercial poultry farms in Ukraine between 2014 and 2016. IBDV genetic material was amplified directly from bursa. The nucleotide sequence for VP2 hypervariable region of 16 IBDVs were determined by RT-PCR method, sequenced and compared to well characterised IBDV isolates worldwide. Neighbor-joining method was used for phylogenetic analyses. In result of the study Ukrainian IBDVs represented two genetic lineages: very virulent (vv) IBDVs and classical IBDV closely related to attenuated vaccine stains. The nucleotide identity among Ukrainian vvIBDVs ranged between 87.2% and 99.8%. Ukrainian vvIBDV strains clustered together with very virulent strains from other countries like: United Kingdom, Egypt, China, Netherlands and Spain. In conclusion this report demonstrates the circulation of vvIBDV in commercial poultry farms in Ukraine.

Keywords: Infectious bursal disease virus, vvIBDV, VP2, RT-PCR, sequencing, phylogenetic analyses.

Introduction

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

[2, 5]. 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 economical impact in poultry industry worldwide [3]. VvIBDV strains have been characterized in many countries, but there were no publications about these strains in Ukraine.

The IBDV genome is divided into segments A and B: segments A (3.4 kb) and B (2.8 kb). The large segment A encodes 4 viral proteins, the two capsid proteins VP2 (48 kDa) and VP3 (32–35 kDa), the viral protease VP4 (24 kDa), and a nonstructural protein VP5 (17–21 kDa), while the smaller segment B encodes VP1 (90 kDa), an RNA-dependent RNA polymerase. Expression/deletion studies have shown VP2 aa positions 206 to 350 to represent a major conformational, neutralizing antigenic domain called hypervariable region (HVR) [2], which includes the most variable region important for cell antigenic and pathogenic variation. Most exchanges of amino acid residues in VP2 occur in the four hydrophilic loops of the viral capsid [3]. These exchanges indicate that selective pressure for the evolution of IBDV is directly focused on the capsid regions that are immediately exposed to the immune system [4]. Most of the modern research has been focused on the VP2 gene [7,8,9].

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.

Materials and methods

For this research bursa samples were collected from infected chickens at the age of 24 – 44 days. RNA was extracted with silica-based method as recommended by supplier (Ribo-sorb, Amplisens). Reverse transcription was performed using a set of random primers (Reverta-L, Amplisens). The obtained cDNA has been used for the PCR. The oligonucleotide primers used in this work designated Bur1F (5'-TCACCGTCCTCAGCTTAC-3' nucleotide position 587-604) and Bur1R (5'-TCAGGATTTGGGATCAGC-3' nucleotide position 1212-1229) designed to amplify the

hypervariable region of VP2 gene according to Bayliss, amplicon size – 643 bp [1]. To increase specificity and sensitivity of the reaction a second set of primers Bur2F (5'-CGCTATAGCGCTTGACCCAAAAA-3', nucleotide position 651 – 673) and Bur2R (5'-CTCACCCAGCGACCGTAACGACG-3', nucleotide position 1179-1202) designed by Kataria et al [9] has been used which allows the amplification of the inner region of the first amplicon obtained after the first round of the amplification using Bur1F and Bur1R primers. The resulting product had the length of 550 bp. First round of amplification was carried out for 1 cycle at 95 °C for 2 min, 36 cycles at 95 °C for 30 s., 52 °C for 30 s., 72 °C for 30 s., and 1 cycle at 72 °C for 2 min. Amplicons obtained from the first reaction have been diluted in 20 times and used for second reaction. Thermal profile for the second reaction was similar except the primer annealing temperature, which was 63 °C. PCR products were visualized in 1,5 agarose gel. For sequencing 552 bp amplicons which contained hypervariable region of VP2 gene were separated from reaction components using the Thermo Scientific GeneJET Gel Extraction Kit. Purified amplicons were sequenced using Bur2F primer by Institute of Molecular Biology and Genetics (NAS, Ukraine). Sequences were analyzed using Mega 6 software. Nucleotide alignment was performed using ClustalW instrument. For phylogenetic analyses partial VP2 sequences of well characterized IBDV strains have been used. Phylogenetic analysis was performed using neighbour-joining method.

Results and discussion

The nucleotide sequence of the VP2 HVR was determined for 16 Ukrainian IBDV isolates. Nucleotide identity between the 16 isolates ranged between 87.2% and 99.8%. Characteristics of the isolates in table 1.

Table 1. Description of IBDV isolates included in this study

Virus isolate	Date of collection	Region of sample collection	Bird age (days)	Phylogenetic group
Ukraine 1517	17.04.2014	Lviv	44	VV
Ukraine 1853	24.04.2014	Cherkasy	48	CV
Ukraine 38_1943	29.05.2014	Cherkasy	38	CV
Ukraine 43_1943	29.05.2014	Cherkasy	43	CV
Ukraine 2045	29.05.2014	Lutsk	34	CV
Ukraine 58	12.06.2014	Ternopil	10	CV
Ukraine 55	17.06.2014	Kiev	26	VV
Ukraine 691_24	23.09.2014	Lviv	24	CV
Ukraine 691_35_4	23.09.2014	Lviv	35	VV
Ukraine 691_35_5	23.09.2014	Lviv	35	VV
Ukraine 760_45_4	23.09.2014	Lviv	45	VV
Ukraine 760_45_5	23.09.2014	Lviv	45	CV
Ukraine 1147	07.08.2015	Lutsk	37	CV
Ukraine 2065	13.11.2015	Kiev	38	VV
Ukraine 934	04.06.2016	Kiev	36	VV
Ukraine 964	04.06.2016	Kiev	36	VV

The phylogenetic relationship between Ukrainian IBDV isolates sequenced in this study and several well characterized vvIBDVs, classical IBDVs, variant strains and attenuated vaccine strains was inferred using the neighbour-joining method (fig. 1). The Ukrainian IBDV isolates sequenced in this study represent two distinct genetic lineages (1) vvIBDV and (2) classical IBDV strains. Eight IBDV isolates were characterized as very virulent, and eight as classical virulent strains (fig. 1). Ukrainian vvIBDV strains were clustered together with very virulent strains from other countries 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. Very virulent isolates formed two subgroups (VV-1 and VV-2) which indicates on different origin of this strains. There is not enough data to conclude direct correlation between phylogenetic distance and geographical distribution of analyzed strains, but vvIBDV strains have been only identified in Kiev and Lviv region. Similar heterogeneity among vvIBDV strains have been shown by Jenberie et al. [7] in Ethiopia. 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). Isolates 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 have been previously vaccinated with IBDV vaccines that contain V877 or MB/5 strain.

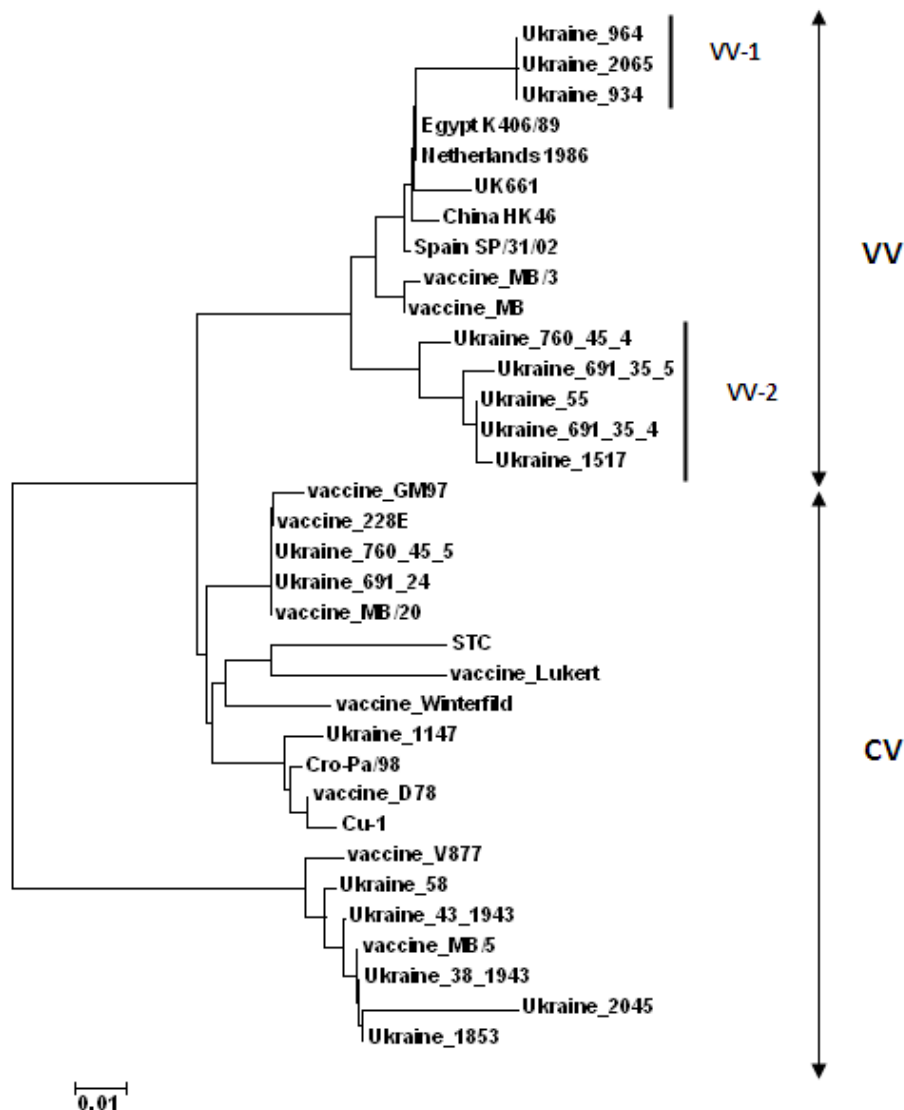


Fig. 1. Phylogenetic analysis of the VP2 hypervariable coding sequence of 34 IBDV isolates. The neighbour-joining consensus tree is shown

In this study we also used sequences of several attenuated vaccine strains to compare nucleotide identity with field isolates. There are three types of vaccine strains: intermediate-plus (or "hot"), intermediate and mild. Despite vaccination outbreaks still occur, that's why it is very important to choose vaccine which is phylogenetically closer to field strain [11,12,13]. The most similar to Ukrainian vvIBDV was MB and MB/3 vaccine strain. This data suggest that level of protection against vvIBDV will be higher than with other vaccine strains used in this study.

Conclusion

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 with intermediate attenuated vaccine strains. Amino acid sequence analyses is needed for further investigation. Information from this study could be used to guide IBDV vaccine selection in Ukraine.

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ГЕНЕТИЧНА ХАРАКТЕРИСТИКА ІЗОЛЯТІВ ВІРУСУ БУРСАЛЬНОЇ ХВОРОБИ НА УКРАЇНІ

Метою дослідження було охарактеризувати ізоляти вірусу інфекційної бурсальної хвороби, що циркулюють у птицефабриках України з 2014 по 2016. Генетичний матеріал було виявлено і ампліфіковано з бурс. Послідовності вірусних РНК було вивалено за допомогою методу ЗП-ПЛР, секвеновано та порівняно із раніше охарактеризованими штамми вірусу ІБХ. Для філогенетичного аналізу було використано метод зближення сусідів. В результаті дослідження віруси ІБХ були представлені двома гілками: високовірulentні штами та класичні віруси ІБХ, близько споріднені з атенуйованими штамми. Гомологія між штамми складала від 87,2% до 99,8%. Високовірulentні штами були близькосторідними із такими виявленими в Великобританії, Єгипті, Китаї, Нідерландах та Іспанії. Висновком даного дослідження є підтвердження циркуляції високо вірulentних штамів вірусу ІБХ в Україні.

Ключові слова: вірус інфекційної бурсальної хвороби, ввІБХ, ЗП-ПЛР, секвенування, філогенетичний аналіз.

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ГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ИЗОЛЯТОВ ВИРУСА ИНФЕКЦИОННОЙ БУРСАЛЬНОЙ БОЛЕЗНИ НА УКРАИНЕ

Целью исследования было охарактеризовать изоляты вируса инфекционной бурсальной болезни, циркулирующих в птицефабриках Украины с 2014 по 2016. Генетический материал был обнаружен и амплифицирован с бурс. Последовательности вирусных РНК были обнаружены с помощью метода ОП-ПЦР, секвенированы и сравнены с ранее охарактеризованными штаммами вируса ИББ. Для филогенетического анализа был использован метод сближения соседей. В результате исследования вирусы ИБХ были представлены двумя ветвями: высоковирулентные штаммы и классическими вирусами ИББ, которые были близкородственными атенуированными штаммами. Гомология между штаммами составляла от 87,2% до 99,8%. Высоковирулентные штаммы были близкородственными с такими выявленными в Великобритании, Египте, Китае, Нидерландах и Испании. Выводом данного исследования является подтверждение циркуляции высоковирулентных штаммов вируса ИББ в Украине.

Ключевые слова: вирус инфекционной бурсальной болезни, ввИББ, ОП-ПЦР, секвенирование, филогенетический анализ.