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FULL GENOME SEQUENCES OF CANINE DISTEMPER VIRUS STRAINS ISOLATED IN KAZAKHSTAN

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Periodic discoveries of dead Caspian seals on the Caspian seashore served as a basis for this study. Various carnivorous animals, basically dogs, foxes and ferrets, are susceptible to canine distemper virus. However, the range of hosts, susceptible to the virus has become wider over the past 20 years. Highly contagious viral disease of carnivores (Carré's disease) is characterized by fever, acute catarrhal inflammation of the mucosa, skin rash, pneumonia, and severe damage to the nervous system. Fatality rate can reach 80-90%. Two canine distemper virus (CDV) strains, namely, Shuskiy strain and Phoca/Caspian/2007 strain have been sequenced and analyzed. DNA sequencing was performed according to Sanger method. Primers were designed using Primer Express 2.0. programme. Morphological properties of isolates were studied with electron microscopy. Cytopathic effect was studied in Vero cell culture. Both strains were isolated from different hosts within a 9 year time period. The first strain was isolated from a mink in 1998 and the second one was isolated from a seal in 2007. A comparison of the nucleotide sequences of both CDV strains has revealed their complete identity. Phylogenetic analysis of whole genome sequences of local strains with other strains from Genbank has showed that local strains form one cluster with Chinese strain, isolated form mink. A phylogenetic analysis of hemagglutinin gene of 79 CDV strains has indicated that both CDV strains belong to the group of vaccine type that is genetically distinct from the Asian and European genotypes.

Keywords: canine distemper virus; phylogenetic analysis; hemagglutinin gene.

INTRODUCTION

Canine distemper virus (CDV) causes highly contagious multisystemic disease of carnivores, which is characterized by fever, acute catarrhal inflammation of mucous membranes, skin eczema, pneumonia and severe damage to nervous system. CDV belongs to genus *Morbillivirus* of the family *Paramyxoviridae* and is closely related to human measles virus and peste des petitsruminantsvirus (An et al., 2008, Appel, 1987, Appel and Summers, B.A., 1995).Over the last 20 years many new morbilliviruses have been identified: phocid distemper virus, dolphin distemper virus and porpoisemorbillivirus. The host range for known viruses has also broadened. Surprisingly, large felids are also susceptible to CDV. This virus was identified in tigers and leopards in California in 1992 (Appel et al., 1994**)** and in lions in Serengeti in 1996 (Plattet, et al., 2005).

Morbillivirus genome is a single stranded negative RNA molecule encoding 6 structural proteins: N (Nucleoprotein), P (Phosphoprotein), and L (Large protein RNA Polymerase-associated protein) (Barrett et al., 1991). There are two independent reading frames in the structure of P-protein gene which encode C and V non-structural proteins. These proteins are the part of the viral replication complex. Multifunctionality of the P gene fragment makes it highly conservative, which allows us to use it as a genetic marker for a morbillivirus genus. Fusion protein (F) and hemagglutinin (H) are the part of the viral envelope and are the main antigens for a host immune system (Barrett et al., 1987). CDV was shown to infect both land and marine mammals. Morbillivirus infections in pinnipeds were firstdescribed in 1988 (Osterhaus et al., 1988). CDV caused an epizootic of Baikal seals (Phocasibirica), and has led to the deaths of 10-15 thousands animals during the period of autumn 1987 and spring 1988 (Grachev et al., 1989).

In 1988 mass mortality of seals (Phocavitulna) has occurred in the seas of Northern Europe. The disease was caused by a strain closely related to CDV morbillivirus which was later described as an independent Phocine distemper virus strain (PDV) (Kennedy, 1990).

Massive mortality of the seals was registered on Caspian Sea during the period of 1997-2007 (Ohishiet. al., 2001). In April- June of 2000 the major episode of seal mortality over the last century has occurred on the Kazakhstani coastal zone of Caspian Sea (Kennedy et al., 2000, Kuiken et al.,2006). Laboratory analyses of the dead seals, as well as an examination of disease patterns had detected a morbillivirus infection. The objective of this study

was to report genetic features of the CDV strains isolated from a Caspian seal (Phoca/Caspian/2007 strain) and a mink (Shuskiy strain)found dead in Kazakhstan in 2007 and 1998, respectively.

MATERIALS AND METHODS

Samples."Shuskiy" and "Phoca/Caspian/2007" CDVstrains were used in this study. Strain "Shuskiy" was isolated from the mink in Zhambyl province, Kazakhstanin 1998. Strain "Phoca/Caspian/2007"was isolated from the seal found on the Kazakhstani coastal zone of Caspian Sea in 2007.

The characteristics of the strains.

The "Phoca/Caspian/2007" canine distemper virus strain was isolated on the Kazakhstani Caspian Sea coast from organs of the dead seals during an epizootic infection of the seals in spring 2007. Organs of the dead seals were collected and stored at – 80^{o}C. Homogenates contained approximately 2 g of pathological material from the dead seals. The virus was isolated during a successive passage in the Vero cell cultures. A monolayer of the Vero cells was infected with the homogenate. The cytopathic effect (CPE) was observed on daily basis (Lan et al., 2005).CDV was titrated in a 10 fold dilution using a 50% tissue culture infectious dose ($TCID_{50}$) assay (Yamaguchi et al., 1988**)**.

The virus was purified and concentrated from the culture suspension by precipitation with PEG-6000. Further purification was achieved in the sucrose density gradient (30-60%).

Identification of the isolated pathogen was performed by ELISA and electron microscopy.

The «Shuskiy» canine distemper virus strain was isolated from organs of the dead minks during an epizootic infection of minks in the fur farms of the Zhambyl region in autumn 1998. The virus was isolated during a successive passage in a primarytrypsinized dog-kidney culture. The isolated pathogen later adapted to the Vero cell cultures.

RNA extraction, RT-PCR, primer design and sequencing

Total RNA was extracted from a purified and concentrated virus using a microcolumn-based QIAamp Viral RNA Mini kit (QIAGEN, USA). The cDNA was synthesized using First Strand cDNA Synthesis kit (FERMENTAS, USA).

The complete genome was amplified in 28 overlapping fragments with primers described in Table 1. The average PCR product size was 600 bp and the average overlap between products, excluding primers, was 35-60 bases.

Table 1. Oligonucleotides used in amplification and sequencing

№	Forward Primer Sequence 5'-3'	Reverse Primer Sequence 5'-3'	Position in the genome	PCR-Product Size, (bp)
1	scdv_1AgACAAAgTTggCTAAggATAg	rcdv_1 ATATATTgAATTGCTCAgCATTATC	4-25 580-604	600
2	scdv_2 AgACATAgAAgTTgATAATgCTgAg	rcdv_2 AgTAAgATCgACCgAAATTTAACC	566-590 1153-1176	610
3	scdv_3 ggTTggTgTTgAACTTgAAAAC	rcdv_3 TAgAATgATAATTgTTgACTgATgC	1121-1142 1698-1722	601
4	scdv_4 TTgCATCAgTCAACAATTATCATTC	rcdv_4CCCAgTTAgATgAAgCATTTCC	1696-1720 2284-2305	609
5	scdv_5 TgATgATAgCACTgAggATTCTg	rcdv_5AATTgAATCAgTCTCTCCTTTAAg C	2244-2266 2823-2847	603
6	scdv_6TACTgCTTAAAggAgAgACTgATTC	rcdv_6AgCggACTTAggCTCTTgTg	2819-2843 3404-3423	604
7	scdv_7CTgCTTTCACTATCgCTTAAAAg	rcdv_7 CTggAgCggAATTCAAACATC	3361-3383 3959-3979	618
8	scdv_8GACTATCAgACgATggAAgTTACAg	rcdv_8 TCTggATggTCggTgCTTATAC	3923-3948 4532-4544	621
9	scdv_9 AgCgAggATTgAgggTATAAgC	rcdv_9TggTgTAggAgACgggTCTg	4507-4528 5096-5115	608
10	scdv_10 CACAACATCAgCTCAgCgATC	rcdv_10gCCACTCCTAAAgCTACACCTg	5048-5068 5626-5647	599
11	scdv_11 gTggTACTTgCAggTgTAgC	rcdv_11gCAATATACCTCgggACAgTg	5616-5635 6209-6229	613
12	scdv_12 ggAAgCAgTTTCTTACAACATAgg	rcdv_12 TAATATAggAACACTgAggAgACTg	6167-6190 6775-6779	612
13	scdv_13 CTTTAATTTTggCAgTCTCCTCAg	rcdv_13ATCTATgACTTggTgATgTACgg	6743-6766 7326-7348	605

14	scdv_14 AgATTgCTgAAAgAggATATggAg	rcdv_14TATAgTTggTTgTTTggAgTAATgg	7292-7315 7868-7892	600
15	scdv_15 AAggTggCTgAATgACATgC	rcdv_15TCCAgAgATTgTTCCgTCTTTg	7849-7868 8462-8484	635
16	scdv_16 CCCAAAgACggAACAATCTCTg	rcdv_16 gAgTCCATggCTAAAAggATCTTg	8438-8460 -9038-9062	624
17	scdv_17 ACAgACgATTgAATTAAACCATCTC	rcdv_17 TTgCgACAgTTgTgAgTAgAg	8964-8988 9572-9592	628
18	rcdv_18 TgAgATCAgTgATTAAATCCTCTAC	rcdv_18gggTTgATTCATgTgTTTCCg	9553-9577 10140-10160	607
19	scdv_19 TTTAgAAgTTTCggTCACCCAAg	rcdv_19 TCgCATTTTgTAggTCATTTTAgC	10086-10108 10668-10691	605
29	scdv_20ATTAAAgAggTAgggAgATTATTCg	rcdv_20ATAgTCCATAACTTCTgACAATACC	10644-10668 11251-11275	631
21	scdv_21 gCCCCTAACTCTCAAATATTCATC	rcdv_21 AgAgACAgTgATCTTgTAAgAgg	11202-11225 11786-11808	606
22	scdv_22 gATgACACgggATgTgATAgAAC	rcdv_22 gCCCTAAATTgCTCATAATCgTAAg	11762-11784 12343-13367	1605
23	scdv_23 ACTTACgATTATgAgCAATTTAggg	rcdv_23 CTgATgAgAgAggTCCCTgAg	12342-12366 12926-12946	604
24	scdv_24 TCACCgACTAAgAgACAAgAgTAC	rcdv_24 gCAgCACATTgACCTAgATATACAg	12890-12913 13480-13504	614
25	scdv_25 CACTgAgTTTCTTCTAgTTgAgC	rcdv_25 gATTgAgCCTCTTCTAAgATATgTC	13445-13467 14042-14066	621
26	scdv_26 TgTTCCCTgACATATCTTAgAAgAg	rcdv_26 TCTggCTCAgTATgTACTTgTAAC	14034-14058 14614-14637	603
27	scdv_27 TTTCAATgggAgACCAgAAgTAAC	rcdv_27 TggTgAAgCAggTTCTTACATAC	14570-14593 15147-15149	579
28	scdv_28 gggCTTACAATTAATggTCTTAAAg	rcdv_28 CAgACAAAgCTgggTATgATAAC	15123-15147 15666-15688	565

PCR amplification was performed in a final volume of 30 ul, containing 3 ul of 10x PCR Buffer (100 mM Tris HCl, pH 8,3, 15 mM $MgCl_2$, 750 mM KCl), 0,6 ul of dNTP (10 mM), 10 pmol primers (forward and reverse), 0,2 ul of Taq DNA polymerase (5U/ul), cDNAup to 1 ng and Milli-Q H_2O up to 30 ul in following conditions: 94°C for 3 minutes, 35 cycles of amplification (94°C for 30 seconds; 50-55°C for 30 seconds; and 72°C for 1 minutes), and a final 10 minutes extension at 72°C. The PCR products were analyzed by 1.0% agarose gel electrophoresis and stained with ethidium bromide. Pieces of the gel containing DNA bands of the expected sizes were purified using Wizard® PCR Preps DNA Purification kit, (Promega, USA) as described in the manufacturer's protocol. The purified DNA was used as a target for the direct nucleotide sequencing using a Cycle Sequencing kit (BigDye Terminator version 3.1; Applied Biosystems), followed by analysis in an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA).

Assembly of the overlapping sequences

The partial nucleotide sequences obtained from the CDV virus were first inspected for the product quality and then assembled bySeqMan II program from the LASERGENE software package (version 6.0; DNAstar,Madison,

WI) in order to produce contiguous full-length sequences. This software was also used to predict the entire open reading frame (ORF) for the proteins.

The nucleotide sequence accession number

The complete nucleotide sequences of the CDV genomes were deposited in GenBank under accession no. HM063009 and HM046486.

Phylogenetic analysis

Complete nucleotide sequences of the Kazakhstani CDV strains were compared to 16different CDV strains. The full-length H gene sequences of the Kazakhstan CDV strains were compared to those of the 77 CDV strains which were isolated from the various animal and geographical origins (Table 2). The Accession numbers for these complete H gene nucleotide sequences were obtained from the GenBank nucleotide database at the National Center for Biotechnology Information (NCBI). These nucleotide sequences were aligned by using the software package Mega version 4.0 (http://www.megasoftware.net).

Table 2. Origins and dates of collection of the 79 reference CDV strains used in this study

№	Strain/Isolate	Country/Year	Host	Accession №
1	Canine/NTU 1-2004	Taiwan, 2004	Dog	DQ191175
2	TW/07-PT18	Taiwan, 2007	Dog	EU296494
3	«20»	China,2008	Dog	GQ332533
4	«24»	China,2008	Dog	GQ332535
5	HLJ(08)3	China,2008	Dog	FJ423606
6	HZ026	China	Dog	EU532600
7	SD(07)1	China,2007	Dog	EU379560
8	BS0610	China,2006	raccoondog	EU934233
9	07Q72	Korea,2007	Dog	EU716072
10	HLJ3-08	China,2008	Dog	FJ409464
11	JL(07)3	China,2007	Dog	EU564812
12	JL(07)4	China,2007	Dog	EU564813
13	ZD01	China	Fox	EF445051
14	HLJ2-07	China,2007	Dog	EU593894
15	«23»	China,2008	Dog	GQ332534
16	JL(07)2	China, 2007	raccoondog	EU325729
17	Canine/NTU 2005-2	Taiwan, 2005	Dog	DQ887548
18	H06Ny13	Hungary,	Dog	DQ889189
19	TW/05-K55	Taiwan, 2005		EU296483
20	Canine/NTU 3-2004	Taiwan, 2004	Dog	DQ191766
21	Monkey-KM-01	China,2008	Monkey	FJ405224
22	Monkey-BJ-01	China,2008	Monkey	FJ405223
23	Ueno	Japan	Dog	D85753
24	Yanaka	Japan	Dog	D85755
25	H05Bp2S	Hungary, 2006	Dog	DQ889178
26	H06Bp7F	Hungary, 2006	Dog	DQ889183
27	H06Bp10S	Hungary, 2006	Dog	DQ189186
28	179/94	Italy, 1994	Dog	DQ26087
29	Liud	China	Dog	AF172411
30	11L	SouthAfrica,2007	Dog	FJ461719
31	5sp	SouthAfrica,2007	Dog	FJ461718
32	97Jindo	Korea,1997	Dog	EU716073
33	HM-3	Japan,1999	Dog	AB040767
34	98Marten	Korea,1998	Marten	EU716074
35	5B	Japan,1999	Dog	AY297453
36	HLJ1	China,2004	Dog	EU743934
37	55L	Japan		AB295485
38	98-002	Japan,1998	dog	AB025270
39	26D	Japan,1999	dog	AB040766
40		China	Giant panda	AF178038
41	PDV-2	Russia	phocaSibirica	X84998
42	1493/Han89		Germanferret	X84999
43	US89	USA,1989	raccoon	Z47765

44	A75/17	USA		AF164967
45	A92-27/4	China	Chineseleopard	Z54156
46	00-2601	USA, 2000	raccoon	AY443350
47	01-2690	USA,	raccoon	AY465925
48	Bruno107Arg2005	Argentina, 2005	dog	FJ011005
49	207/00	Italy, 2000	dog	DQ228166
50	Danishmink	Denmark	mink	Z47759
51	H06Ny11	Hungary, 2006	dog	DQ889187
52	H06Ny13	Hungary,	dog	DQ889189
53	Greenlandicdog	Denmark	dog	Z47760
54		Turkey	dog	AY093674
55	265/02-3	Italy	dog	DQ494318
56	111/03B	Italy	dog	DQ494319
57	2544	Germany,1997	dog	Z77672
58	Danishdog"	Denmark	dog	Z47761
59	324/03	Italy	dog	DQ494317
60	5804/Han90	Germany	dog	X85000
61	5804P			AY386316
62	Pao2003Arg90	Argentina, 2003	dog	FJ011004
63	KDK-1	Japan	dog	AB025271
64	Vn86	Vetnam,2009	dog	AB472690
65	Vn99	Vetnam,2009	dog	AB472691
66	Isolate Onderstepoor			AF014953
67	Lederle	Hungary, 2006	dog	DQ903854
68	Vaccine B	Africa, 2006		FJ461709
69	Convac vaccine			Z35493
70	98-2666-2	USA,1998	raccoon	AY548111
71	98-2646	USA, 1998	raccoon	AY542312
72	98-2645	USA, 1998	raccoon	AY445077
73	98-2654	USA, 1998	raccoon	AY466011
74	CDV3	China,2006		DQ778941
75	Snyder Hill	Germany	raccoon	AF259552
76	MD77	Japan, 1970	dog	AB286953
77	Phoca/Caspian/2007	Kazakhstan,2007	seal	HM046486
78	Shuskiy	Kazakhstan,1989	mink	HM063009
79	PDV/DK88-4a	Denmark		AF479276

The phylogenetic analyses were conducted using the MegAlignprogram from the LASERGENE software package (version 6.0; DNAstar,Madison, WI) and with Mega version 4.0 software. A multiple alignment was created using Clustal W software,and the neighbor-joining method was used for the constructionof the phylogenetic tree.

RESULTS ANDDISCUSSION

Virus isolation

The characteristics of cytopathic effect (CPE) were determined in Vero cells. We have isolated a cytopathogenic agent (strain Phoca/Caspian/2007) after 3 passages in Vero cells. Both strains caused syncytium CPE in Vero cells. The highest titer for the Vero cell-associated virus of the Phoca/Caspian/2007 strain was 5,5lg $TCID_{50}$/ml (tissue culture infectious dose). The maximum infectivity titer of the Vero cell-associated virus of Shuskiy strain was 5 lg $TCID_{50}$/ml.

The electron microscopic study of the seal isolated viral specimenhas allowed us to conclude that morphology and structure of ribonucleoproteins (RNP) and virionsbear a strong resemblanceto theparamyxovirus infection. The diameters of the round intact virions in the purified preparations were 300-400 nm and the diameter of RNP was 18-20 nm (Fig. 1).

Биотехнология. Теория и практика. 2013, №3, стр. 11-19
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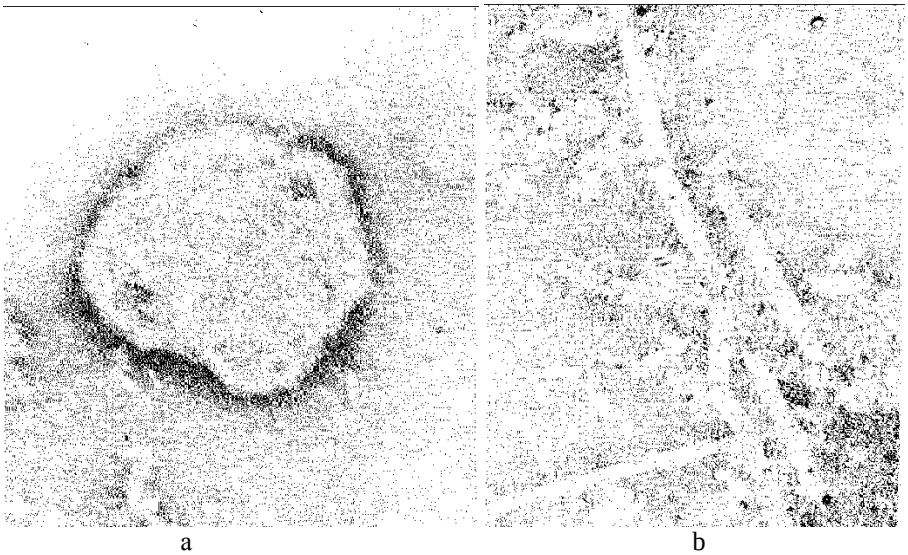

a b

a – virion; b – ribonucleoproteins

Fig. 1. Electron micrograph of the canine distemper virus isolated from seals. Negatively stained with the 2% phosphotungstic acid, pH 7.0 x100,000

Sequence analysis of viral genomes

The complete genome sequences of the CDV (15690 bp) were obtained by the direct sequencing of the 28 overlapping PCR fragments. The Phoca/Caspian/2007 and Shuskiystrains have the same genome organization as the other known genomes of CDV. The genome sequence is 15690 nucleotides long and encodes the same set of proteins (N, P, M, F, H and L) as the CDV virus type does. The predicted entire open reading frames (ORF) of the viral proteins are shown in Table 3.

Table 3. Genome organizations of Phoca/Caspian/2007 and Shuskiy Kazakhstan strains of CDV (protein, size, ORFs)

Gene	Protein	ORF (nucleotideposition)
N-gene	nucleoprotein (NP)	108….1679
P-gene	phosphoprotein (P)	1801….3324
-	C protein	1823…2347
M-gene	matrix (M) protein	3432..4439
F-gene	fusion (F)	4935…6923
H-gene	Haemagglutinin(H)	7079….8893
L-gene	Large (L) protein	9030….15584

The complete nucleotide sequences of Phoca/Caspian/2007 and Shuskiy Kazakhstani strains of the CDV were aligned and compared with the reference nucleotide sequence of the CDV (NC_001921). Nucleotide sequence analysis indicated 100 % homology between the Kazakhstani strains of CDV.

The complete nucleotide sequences of the 16 CDV complete genome sequenceswere aligned with the complete nucleotide sequences of the Phoca/Caspian/2007 and Shuskiy strains using MEGA software (version 4.0). The accession numbers for these complete nucleotide sequences were obtained from the GenBank. Sequence alignment was used to create a phylogenetic tree (Fig. 2).

Биотехнология. Теория и практика. 2013, №3, стр. 11-19
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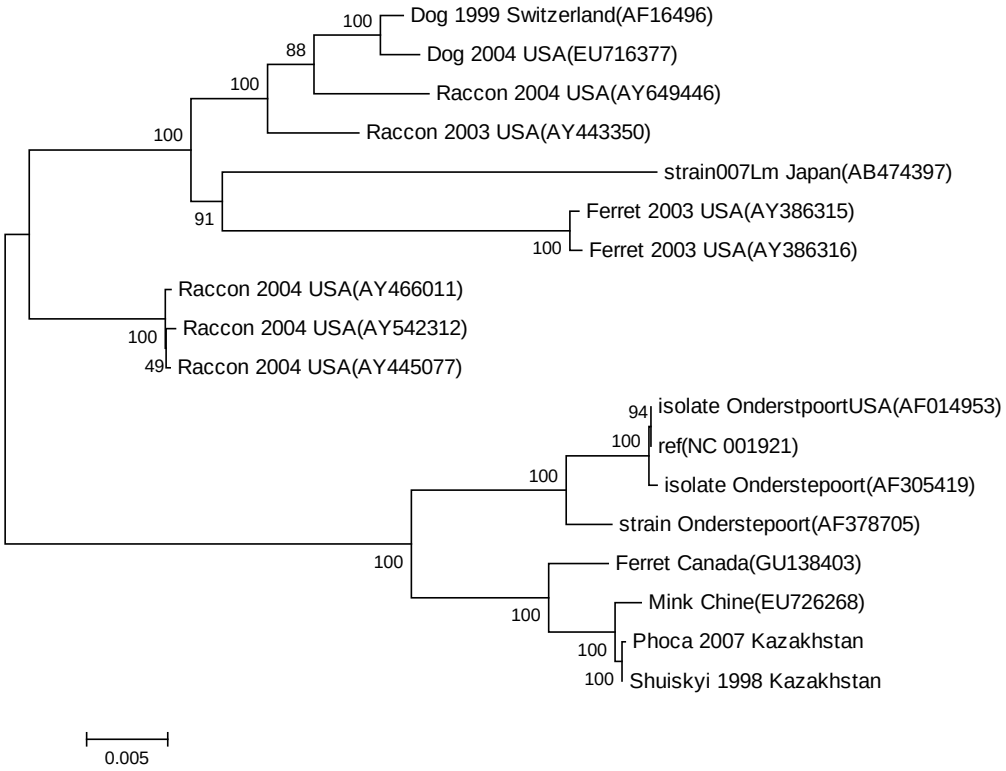

Fig. 2. The phylogenetic relationships of the 16 CDV strains and Kazakhstani CDVstrains based on the complete nucleotide sequences. The distance values were calculated by the ClustalW program within the MEGA 4.0 software package. The Neighbor-Joining algorithm was used to generate the tree. Bootstrap values were calculated on 1000 replicates

The phylogenetic analysis has showed that the sequences of the Kazakhstani CDV strainsform a single cluster (Fig. 2). These strains formed a cluster with the vaccine strain CDV3 (EU726268), isolated from the mink in China.

The predicted amino acid sequences of the Kazakhstani strains were compared withrepresentative sequences of vaccine and virulent strains of the CDV. The amino acid identity of the complete amino acid sequences of the two strains with theOnderstepoort (AF378705) vaccine strain was 96% and with 5804P (AY386316) virulent strain was 92%.

The H genes of the two Kazakhstani strains consisted of 1821 nucleotides in one open reading frame encoding 607 amino acids. The complete nucleotide sequences of the H gene of two Kazakhstani strains were compared to 77 CDV strains. The appropriate Phocine distemper virus sequence (PDV, DK88-4a) was included for the outgroup rooting. The phylogenetic analysis of the CDV strains sequences is shown in Fig. 3.

Биотехнология. Теория и практика. 2013, №3, стр. 11-19
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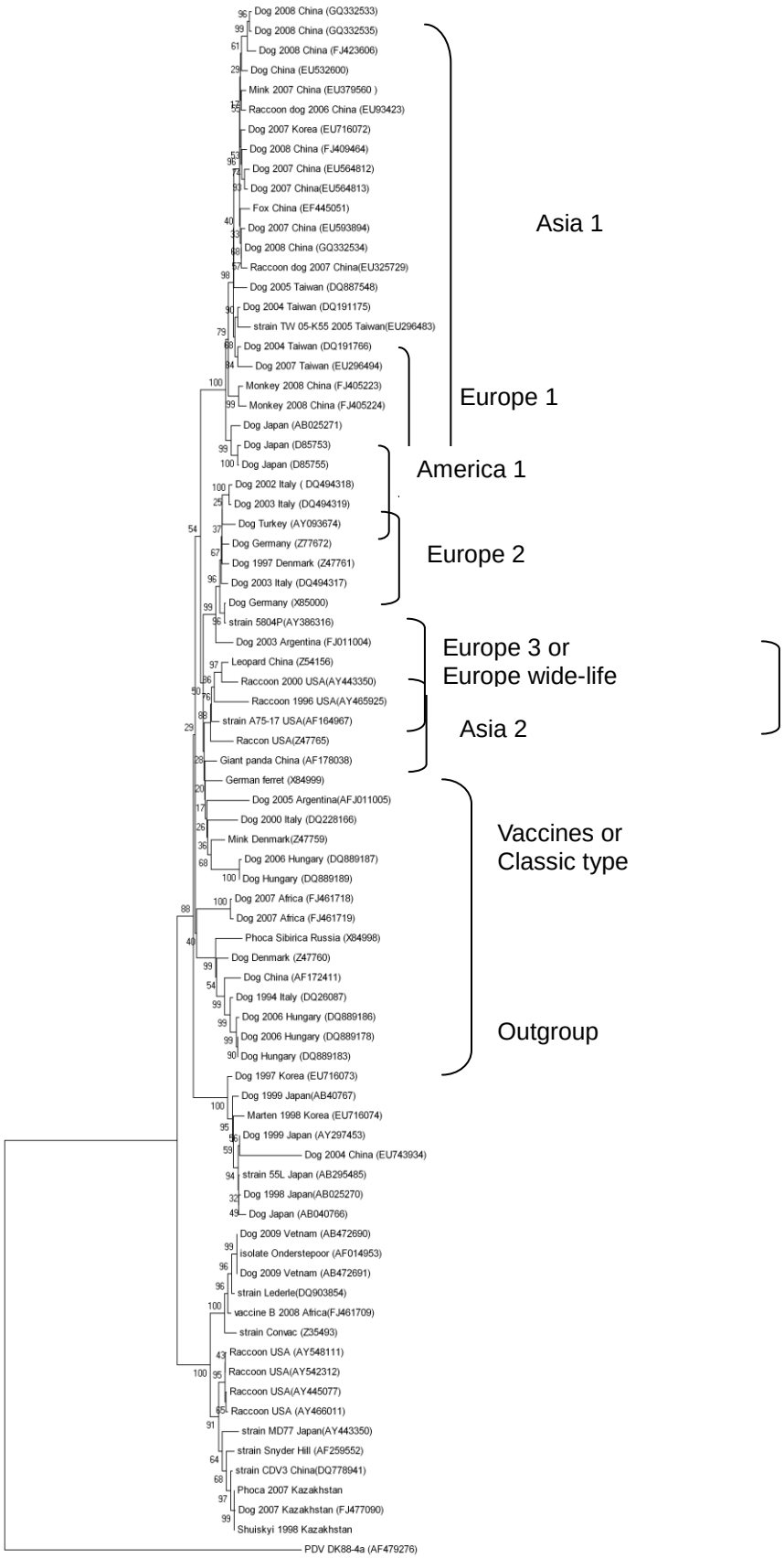

Fig. 3. Phylogenetic analysis of the H gene sequences from different strains. The sequences of 77 CDV strains were compared, including two from this study. Distance values were calculated by the Clustal W within the Mega 4.0 software package. A phylogenetic tree was constructed from the aligned sequences by neighbor-joining (NJ) method. Bootstrep values were calculated on 1000 replicates

Биотехнология. Теория и практика. 2013, №3, стр. 11-19
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As a result,Kazakhstani Phoca/Caspian/2007 and Shuskiystrains were classified into the vaccine or classic group that is genetically distant from the Asian groups. Both CDV strains, Phoca/Caspian/2007 and Shuskiy, have a 100% homology of the H protein. The old CDV strains, such as Onderstepoort, Convac, Leder and Snyder Hill also belong to the group of the classic or vaccine type.

Both strains contained the potential asparagine (N)-linked glycosylation sites [N-X-S/T] at the following positions: amino acids 19-21, 149-151, 391-393, 422-424, 456-458, 587-589, 603-609 (Fig. 4).

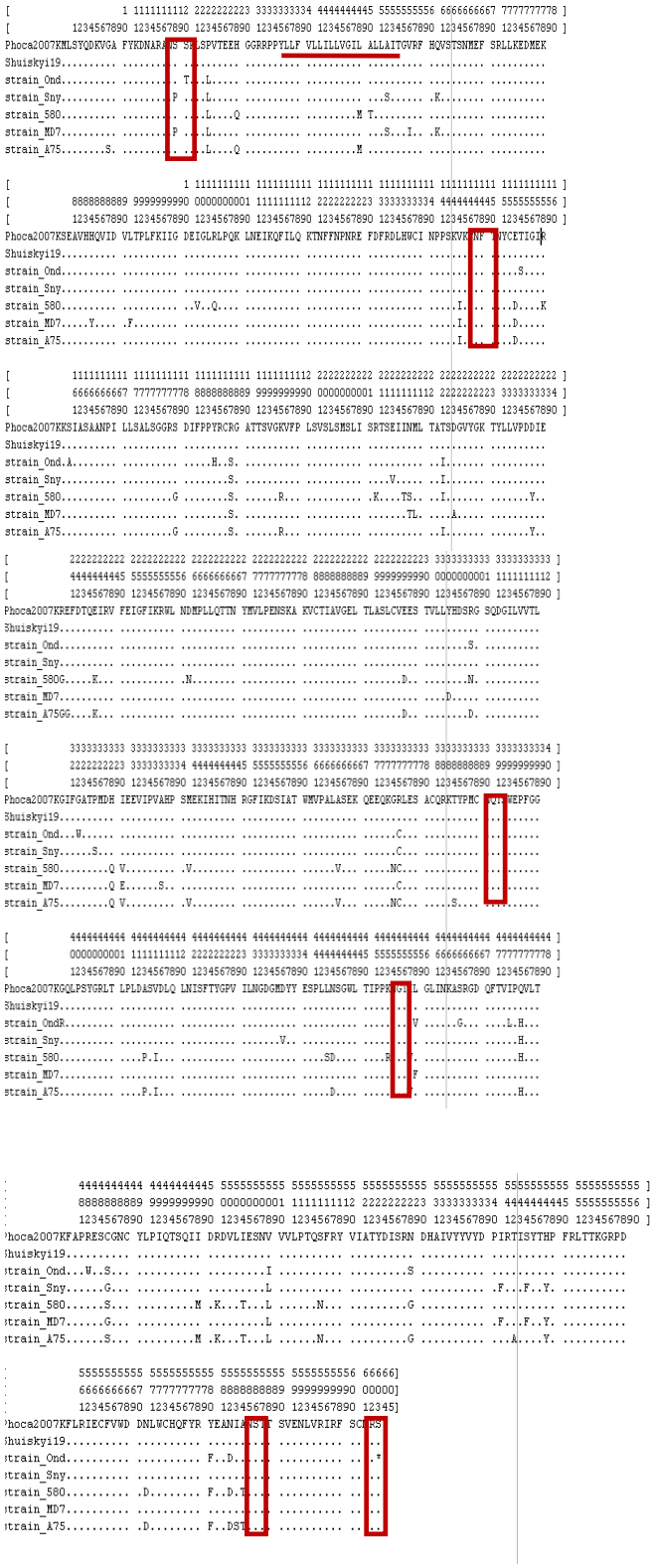

Fig. 4. Alignment of the predicted H protein amino acid sequences of two Kazakhstani CDV strains and other reference strains from the Genbank. These sequences were aligned using MEGA 4.0 software package. The major N-terminal hydrophobic region (amino acids 35–55) is uderlined. The potential glycosylation sites are boxed. Dots represent identical amino acids

In this study we report the whole genome sequences of the Kazakhstani CDV strains. Comparative analysis of the sequencesof Kazakhstani CDV strains has showed high degree of similarity in length and genetic organization comparing to known CDV sequences. A comparison of the nucleotide sequencesofShuskiy and Phoca/Caspian/2007CDV strains has revealed their complete identity.

It appears that since 1988 an expansion of the already broad natural host spectrum of CDV has occurred. The recent outbreaks of distemper in minks (Cunningham et al., 2009), raccoon dogs (Lednicky et al., 2004) raccoons (Lednicky et al., 2004), ferrets (Williams et al., 1988), foxes (Martella et al., 2002) lions and jaguars (Appel et al., 1994, Roelke-Parker et al., 1996) pandas (Qin et al., 2010) and seals in Lake Baikal (Grachev et al., 1989, Kuiken et al., 2006) have underscored the ability of CDV to invade new host species.

Cellular receptors are one of the major determinants of the host range and tissue tropism of a virus. Signaling lymphocyte activation molecule (SLAM) is thought to be a major cellular receptor for high-host specificity of morbilliviruses, which cause devastating and highly infectious diseases in mammals. It has been reported that SLAM (also known as CD150) is a cellular receptor for morbilliviruses including CDV (Tatsuo et al., 2001).Ohishiet al. (2010) have determined the sequences of SLAM cDNA from five species of marine mammal, including two cetaceans, two pinnipeds and one sirenian, and generated three-dimensional models to understand the receptor–virus interaction. Twenty-one amino acid residues in the immunoglobulin-like V domains of the SLAMs were shown to bind the viral protein. Notably, the sequences from pinnipeds and dogs were highly homologous, which is consistent with the fact that canine distemper virus was previously shown to cause a mass die-off of seals (Ohishi et al., 2010).

Morbillivirus attachment to susceptible cells occurs via interaction between SLAM and the viral hemagglutinin (H) protein, one of two glycoproteins that are inserted into the viral membrane and subsequently expressed on the surfaces of infected cells. The H protein is the major determinant of tropism and cytopathogenicity and is useful for phylogenetic analyses. Based on complete H gene sequences, CDV isolates havebeen divided into eight distinct geographically separated genotypesor lineages Europe 1, Europe 2, Europe 3, North America 1, North America 2, Asia1, Asia 2 and Vaccines, but some relationships among these lineages remain ambiguous (An et al., 2008).

Jian-Jun Zhao et al. (2010) had analyzed the hemagglutinin gene sequences of 28 Chinese CDV strains isolated from the different animal hosts in 2004-2008 (Zhao et al., 2010). The 28 Chinese CDV sequences were compared to other 67 CDV strains. The Chinese wild-type viruses (26/28) were grouped together in one branch in the Asia-1 genotype. In this genotype, other Chinese CDV strains identified from dogs or other wild-life species after the 1990s were also included, along with some Japanese and Korean strains detected before 1998. The sequences of the 26 Chinese CDV strains were approximately 96,1–99,5% identical to each other, but they were isolated from different animal species (Zhao et al., 2010).

Strains Shuskiy and Phoca/Caspian/2007 were isolated from the seal and the mink that had not been given a vaccine against CDV infection. Phylogenetic analysis showed that the Kazakhstani CDV strains do not belong to Asian type. The phylogenetic analysis showed that both strains belong to the group of vaccine or classic type of CDV. Both Kazakhstani strains are in the same subcluster with CDV3 vaccine strain which was isolated in China. The group of vaccine or classic type of CDV contains Onderstepoort(Lan et al., 2006**),**Lederle(Demeter et al., 2007), Convac1**(**Appel et al., 1994), 98-2654 vaccine strains,MD77 (Hirayama et.al, 1986) Snyder Hill strains (McCulloughet al., 1974).Jian-Jun Zhao et al., 2010 had classified these strains into America-1 genotype (Zhao etal., 2010**)** .America-1 CDVs have been long consideredextinct but recent studies have demonstrated that they still circulate in raccoons in America and in dogs in Thailand and Vietnam (Lan et al., 2009, Lednicky et al., 2004). Lan*et al.* (2009) showed that the Vietnamese strains belong to the group of vaccine strains and classical strains which is genetically far from Asia 1 and Asia 2 groups (Lan et al., 2009). Over the last five decades, CDV isolates from the latter lineage, such as Onderstepoort, and Snyder Hill, were applied in vaccine production and used as conventional distemper vaccines (Chulakasianet al., 2010).

The nucleotide sequence of H gene of the two Kazakhstani CDV strains showed identity of 97,7, 96,9, 90,6, 99,7, 97,7 and 90,6% when compared with Onderstepoort, Leder, Convac, CDV3, MD77 and Snyder Hill, respectively. Onderstepoort has been used as a reference or a vaccine in many laboratories (Lanet al., 2005). Strain MD77 is a virulent strain and caused a severe disease in dogs in the 1970s in Japan(Hirayama et al., 1986). Strain MD77 belongs to the classical type. Identities of complete genome of CDV between Kazakhstani CDV strains and CDV3 were 99,8% and between Kazakhstani CDV strains and Snyder Hill were 99,2%. The Snyder Hill strain has a high virulence and causes acute encephalomyelitis with predominantly grey matter changes in dogs (McCullough et al., 1974).Onderstepoort, MD77 and Snyder Hill are Old CDV strains, all which were isolated in 1930–1950s.

The conformational change of the H protein conducts a signal to the fusion (F) protein, which then mediates fusion between the viral and host cell membranes. However, the extent and efficiency of cell-cell fusion is H protein-dependent (von Messlinget al., 2001, Sawatsky B. and von Messling V et al., 2010).Post-translational modifications, including N-linked glycosylation, are essential for the function of many integral membrane proteins

and secreted proteins.Sawatsky& von Messling (2010) first determined the role of decreased natural N-glycosylation of the vaccine strain H protein in protein function and virulence (Sawatsky B. and von Messling V et al., 2010). Onderstepoort vaccine strain lacks N309S, T393A and N456D potential glycolisation sites, which are present in 5804P, A75/17 virulent strains and in Snyder Hill strain belong to the group of classical strains. Both Kazakhstani strains lacked the potential glycosylation sites at positions 309-311 and 584-586. N-glycosilation site at position 584-586 is predominant in Asian strains (Iwatsukiet al, 1997).

It was shown that adaptation of CDVs to Vero cells causes loss of pathogenicity (Sekiet al., 2003) and changes growth characteristics due to cytopathic effect (CPE) (Plattet et al., 2005). Sequence comparison of Vero-adapted A75/17-V strain that was passaged 17 times in Vero cells with wild-type strain A75/17 showed seven nucleotide changes: at nucleotide positions 2275, 2381 and 2399 in the P/V/C gene, at nucleotide positions 3610, 4422 and 4434 in the M gene and at nucleotide position 14940 in the L gene (Plattet et al., 2005).

In this study two CDV strains were isolated from the unvaccinated animals. CDV vaccination is not routinely given to animals in Kazakhstan. Due to this fact, the mutation rate of the viral genome was relatively low. It is quite possible that the virus still circulates in some areas of the Republic of Kazakhstan. Probably, Kazakhstani strains still keep the original characteristics of the classic type CDV. However, there is evidence that virulence for the natural host may be lost when CDV is adapted to cell culture (Plattet et al., 2005).Future studies are needed to show if this is true for local Kazakhstani CDV strains.

CONCLUSION

In conclusion, two CDV strains isolated in Kazakhstan belong to the group of classical strains (Old CDVs) which is genetically distinct from other groups (Asian and European). An intriguing characteristic of these genetically identical CDV strains is ability to continually replicate in a group of apparently different hosts, potentially providing a reservoir for CDVepizootic.

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АБСТРАКТ

Основанием для изучения послужили периодические выбросы мертвых особей каспийского тюленя на побережье Каспийского моря. К вирусу чумы плотоядных восприимчивы различные плотоядные животные, в основном собаки, лисицы, хорьки. Однако за последние 20 лет круг хозяев, восприимчивых к вирусу,

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значительно расширился. Высококонтагиозная вирусная болезнь плотоядных животных (болезнь Карре) характеризуется лихорадкой, острым катаральным воспалением слизистых оболочек, кожной экзантемой, пневмонией и тяжелым поражением нервной системы. Летальность может составлять 80-90%. Два штамма (Шуйский и Phoca/Caspian/2007) от тюленя были просеквенированы и проанализированы. ДНК-секвенирование проводили по методу Сенгера. Праймеры подбирали, используя программу Primer Express 2.0. Морфологические свойства изолятов изучали с помощью электронной микроскопии. Цитопатический эффект изучали на культуре клеток Vero. Оба исследуемых изолята были изолированы от разных хозяев с периодов в 9 лет: первый от норки в 1988 г. и второй от тюленя в 2007 г. Сравнительный анализ нуклеотидных последовательностей обоих штаммов вируса чумы плотоядных показал их высокую идентичность. Филогенетический анализ полногеномных последовательностей казахстанских штаммов с существующими в Genbank показал, что казахстанские штаммы образуют один кластер с китайским штаммом, выделенным от норки. Филогенетический анализ гена гемагглютина 79 штаммов вируса чумы плотоядных показал, что оба штамма принадлежат к группе вакцинных штаммов, которые генетически удалены от генотипов азиатских и европейских.

Ключевые слова: вирус чумы плотоядных, филогенетический анализ, ген гемагглютинина.

ҚАЗАҚСТАНДА ОҚШАУЛАНҒАН ЖЫРТҚЫШ ОБАСЫ ВИРУСЫН ТОЛЫҚ ГЕНОМДЫ СЕКВЕНИРЛЕУ

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ТҮЙІН

Зерттеу үшін каспий итбалығының өлі дарақтарының мезгіл-мезгіл Каспий теңізінің жағалауына шығарылып тасталуы негіз болды. Жыртқыштар обасының вирусын әр түрлі етқоректі жануарлар, негізінен иттер, түлкілер, күзендер қабылдағыш. Алайда соңғы 20 жылда вирусты қабылдағыш қожайындардың өрісі біршама кеңейді. Жыртқыш жануарлардың жоғары контагиозды вирустық ауруы (Карре ауруы) безгек, сілемейлі қабықтың асқынған талаураған қабынуы, тері экзантемасы, пневмония және жүйке жүйесінің ауыр зақымдануымен сипатталады. Өлім-жітім 80-90% құрауы мүмкін. Итбалықтан алынған екі штамм (Шуялық және Phoca/Caspian/2007) секвенирленді және талданды. ДНҚ-секвенирлеу Сенгер әдісі бойынша жүргізілді. Праймерлер Primer Express 2.0 бағдарламасын қолданып таңдалды. Изоляттардың морфологиялық қасиеттері электрондық микроскопияның көмегімен зерттелді. Цитопатикалық нәтиже Vero жасушаларының өсіріндісінде зерттелді. Зерттелген екі изолят та 9 жыл кезеңге әр түрлі иелерінен оқшауландырылды: бірінші 1988 жылы қара күзеннен және екіншісі 2007 жылы итбалықтан. Жыртқыш обасы вирусының екі штаммының нуклеотидтік жүйелілігін салыстырмалы талдау олардың жоғары деңгейдегі ұқсастығын көрсетті. Қазақстандық штаммдардың толық геномдық жүйеліліктерін Genbank-тағылармен филогенетикалық талдау қазақстандық штаммдардың қара күзеннен бөлініп алынған қытайлық штаммен бір кластер түзетіндігін көрсетті. Жыртқыш обасы вирусының 79 штаммының гемагглютин генін филогенетикалық талдау екі штамм да азиялық және еуропалық генотиптерден генетикалық алыстатылған вакциналық штаммдар тобына жататындығын көрсетті.

Негізгі сөздер: жыртқыш обасы вирусы, филогенетикалық талдау, гемагглютинин гені.