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MOLECULAR GENETIC ANALYSIS OF RABIES VIRUS IN THE EAST KAZAKHSTAN REGION

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Abstract

Phylogenetic study of genomes of field isolates of lissavirus extracted from the territory of East Kazakhstan is presented in the article in order to carry out sequencing analysis. Ten samples purified PCR products containing rabies virus were examined. As a result of sequencing analysis, ten complete sequences of rabies virus nucleoprotein from East Kazakhstan region were grouped into 6 genotypes, which belong to two lyssavirus groups: Arctic-like group and the most common group - the Steppe group.

Keywords: rabies, PCR, sequencing, phylogenetic tree, strain, isolate, genotype

Introduction

Rabies is an infectious neurological disease of mammals which is almost all cases fatal once clinical signs appear. Lyssavirus causes specific encephalitis (inflammation of the brain) and is the only virus of Vira kingdom, which affects all warm-blooded animals, including humans, with the lethality rate of 100% [1]. The virions are bullet-shaped with an average length of 180 nm and a diameter of 75 nm. The virion consists of an unsegmented genome represented by a single molecule of helically twisted negative RNA and five structural proteins. The rabdovirus genome is represented by a negative polarity RNA molecule and has 5 open reading frames arranged in the genome in the following order: 3'-N-P-M-G-L-5' [2]. Phylogenetic analysis based on the nucleotide sequence of the glycoprotein allowed differentiating representatives of the genus Lyssavirus into seven genotypes [3].

Rabies is an emergent infection considered to be an object of constant increased attention worldwide and is monitored by international organizations (WHO, FAO, OIE). Rabies is the 10th most significant cause of death in the structure of infectious diseases and is registered in

more than 150 countries, according to WHO [4].

The territory of the Republic of Kazakhstan is endemic for rabies - every year hundreds of cases are registered among animals. In 1914, the first case of rabies in Kazakhstan was registered in the Turgai region. And since then the disease has been registered every year. The incidence of rabies among animals and people has not decreased over the past 15 years. During this period of time 1232 unfavorable points were registered, where 1653 animals fell ill [5].

Molecular genetic analysis of rabies, assessment of antirabic immunity, assessment of oral antirabic vaccine eatability methods are used in the global practice, many of them have not been used in Kazakhstan to date or required adaptation and improvement [6].

The sequencing of rabies virus genes will make it possible to advance in the study of the molecular structure and mechanisms of virus variability. Since the use of molecular genetic methods in the diagnosis of rabies will contribute to improving the efficiency of rabies epidemiological surveillance activities [7,8].

Every year in Kazakhstan rabies cases of animals are registered, nevertheless the data on genetic diversity are absent in the literature. The purpose of this work is to obtain data on the genetic diversity of the rabies virus circulating in the East Kazakhstan region and in comparison with the reference strains presented at the National Center for Biotechnology Information (NCBI).

The data obtained would contribute to the development of molecular epizootology, as well as allow to better comprehend the evolution of rabies virus and identify the geographical features of circulating genotypes.

Materials and methods of research

Sampling and RNA extraction

The work material was 10 isolates of Rabies lyssavirus extracted from the brain of different species of animals with clinical signs typical of rabies. All isolates were extracted from East Kazakhstan region in 2021. The samples were screened using a detection kit "FBioNucleo" (Fractal BIO LLC, St. Petersburg, RF) to confirm rabies infection.

Since the brain, which synthesizes a large amount of RNA, was used as material, a reverse transcription strategy with specific primers was adopted. The course of the study included the three steps: choice of primers for reverse transcription and PCR reaction, staging of reverse transcription, and evaluation of the efficiency of synthesis cDNA by PCR and nucleotide sequencing.

N gene reverse transcription

The reverse transcription performed using a Revert-L kit (FBSN Central Research Institute of Epidemiology, RF) to obtain complementary DNA on the RNA matrix. Sens primers at a concentration of 5 pM used as inoculants in the reverse transcription.

Primers were used for N gene amplification (Table 1).

Table 1 - Primers used for N gene amplification

Title	Sequence
Rab-for_1	ACGCTTAACAACCAGATCAAAGAA
Rab-rev_1000	ATCCTACAAAGTGAATGAGATTGAACAC
Rab-for_895	TTCGAGGAAGAGATAAGGAGAATGTT
Rab-rev_2230	GCTTCTTTAACTATGTCATCAAGGTTCAT

Reaction mixture composition for PCR included: 1×Platinum II PCR buffer, 0.2 mM dNTP, 0.2 μM forward and reverse primers, 1 Unit Platinum II Taq Hot-Start DNA polymerase, 3 μL cDNA. The PCR amplification program included: long denaturation at 94ºC - 2 min; 10 cycles: 94ºC - 15 s, 58ºC - 30 s, 68ºC - 1 min; 25 cycles: 94ºC - 15 s, 58ºC - 15 s, 68ºC - 1 min; final elongation 15 min at 68ºC.

PCR products were purified using AMPure XP magnetic particles (Beckman Coulter).

Sequencing of the N gene

Sequencing performed using BigDye Terminator v3.1 Cycle Sequencing Kit. Phylogenetic tree construction was performed in MEGA X using the Maximum Likelihood method and the T92 + G model (Tamura 3-parameter model with gamma distributed).

Results

Overall in 2021 material was collected from animals with clinical picture characteristic for rabies from East Kazakhstan region (Table 2).

Table 2 - Data of collected biological material (positive cases)

No	Name of region, district, village	Sample name	Animal type	Indicator name	Test report number	Name of the sample sequence	PCR Threshold Cycle
1	East Kazakhstan region, Ayagoz district, Kokpekty rural district	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-2-1	21
2	East Kazakhstan region, Ayagoz district, Ayagoz city.	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-3-1	25
3	East Kazakhstan region, Ayagoz district, Ayagoz city.	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-4-1	30
4	East Kazakhstan region, Ayagoz district, Ayagoz city.	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-5-1	28
5	East Kazakhstan region, Ayagoz district, Bidayik rural district	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-6-1	21

6	East Kazakhstan region, Beskaragai district, Beskaragai rural district	Brain	Dog	Rabies	1-S21L-93/1-1	Rab-9-1	25
7	East Kazakhstan region, Beskaragai district, Beskaragai rural district	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-10-1	36
8	East Kazakhstan region, Ayagoz district, Saryarka rural district.	Brain	Fox	Rabies	1-S21L-93/1-1	Rab-11-1	34
9	East Kazakhstan region, Zharma district, Arshali rural district	Brain	Cattle	Rabies	1-S21L-93/1-1	Rab-14-1-blue	35
10	East Kazakhstan region, Ayagoz district, Saryarka rural district.	Brain	Fox	Rabies	1-S21W-94/1-63	Rab-16-6	30

RNA extracted using the R Neasy Mini Kit and a real-time detection PCR was performed to confirm the presence of RNA in the samples. As a result, the presence of lyssavirus RNA was detected in 10 of 15 samples, as indicated by threshold cycle values ranging from 20 to 36 (Table 2). The threshold values were used for genotyping efficiency.

Some 380 full-genome Rabies lyssavirus sequences were imported from the NCBI database in order to find homologous regions between rabies virus genomes. Imported sequences are described with the length from 10015 pairs of nucleotides to 11966 pairs of nucleotides, including: all genomes, isolated from Russia and China, from each country of Europe and the near abroad on 10 genomes, and from each country of far abroad on 3 genomes.

This work resulted in the formation of a collection of cDNA samples from 10 animals with established rabies diagnosis. The cDNA samples were used for genotyping by determining the nucleotide sequence of N genes.

As a result of comparison of 10 field isolates of rabies virus strains by nucleotide sequences of N-gene fragment (figure 1), a dendrogram reflecting phylogenetic relations of strains and isolates of rabies virus in the territory of East Kazakhstan region was constructed.

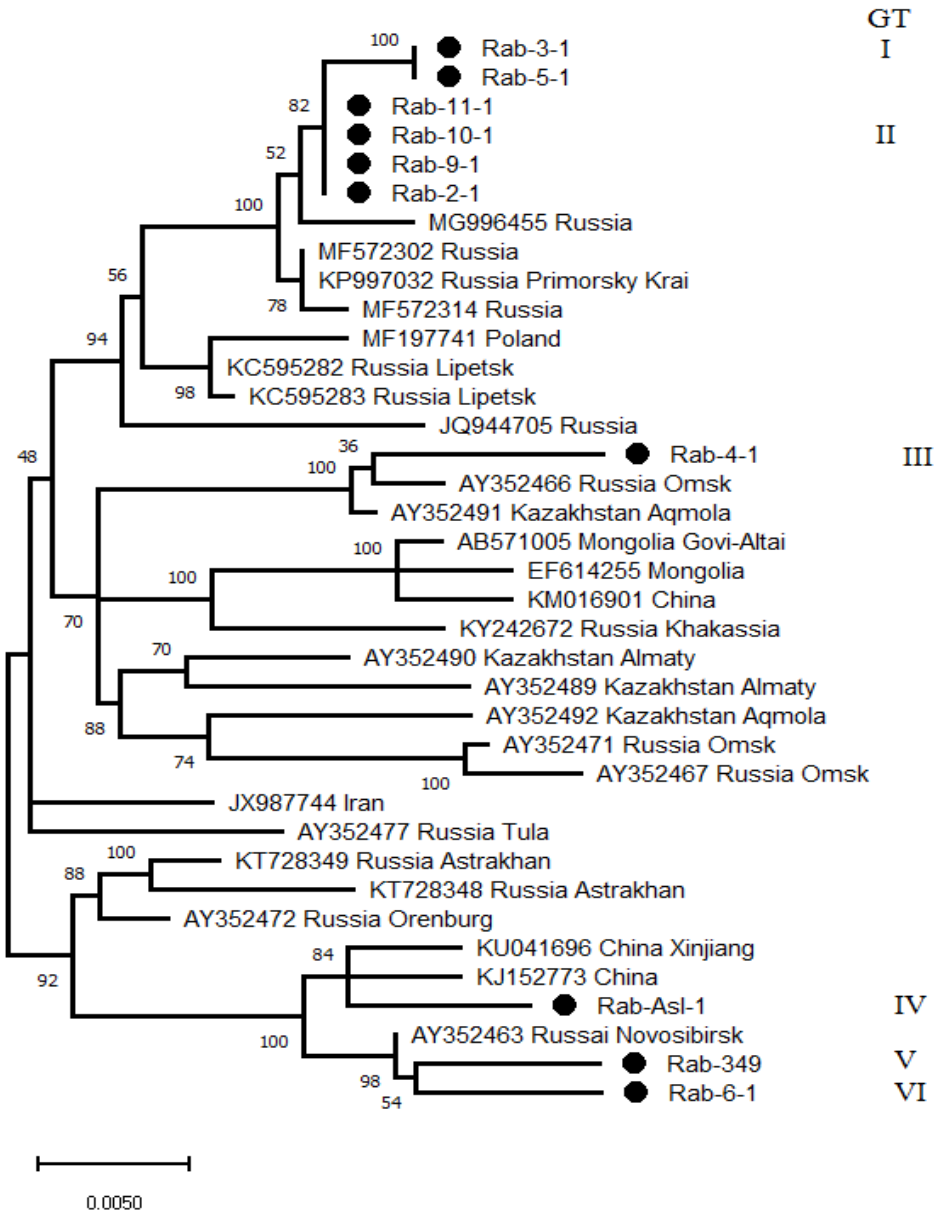

Figure 1 - Phylogenetic tree of rabies virus strains and isolates based on the nucleotide sequences of the N gene fragment

In accordance with the results of the study, all rabies virus isolates studied belonged to the first genotype - Rabies virus.

An analysis of the phylogenetic tree obtained on the basis of N gene fragments (Figure 1) established that the studied isolates can be divided into 6 genotypes. The genotype distribution of isolates in most cases correlates with their geographical origin. For example, phylogenetic analysis grouped 10 complete sequences of rabies virus nucleoprotein from East Kazakhstan region into 6 genotypes. Genotypes 1 and 2, combining 2 and 4 sequences clustered with samples from Russia including sequences from Primorsky Krai. Genotype 3 is the most phylogenetically similar to genotypes circulating in the Omsk Region, as well as in the Akmola Region. Genotypes 4-6 are phylogenetically closer to genotypes circulating in China and Novosibirsk region.

Discussion of the results and conclusion

The first stage included research work, focusing on the collection of pathological material (brain of rabid animals) from the territories of East Kazakhstan region, in which cases of rabies animals were registered. Selection of biological material (brain) from 15 animals with clinical picture characteristic for rabies animals was conducted.

The second stage included formation of a collection of cDNA samples isolated from the brain suspension of animals with a confirmed diagnosis of rabies. To this end, RNA extraction from 15 brain samples was performed, and the presence of rabies virus RNA was established in 10 of them by PCR method. For reverse transcription and PCR amplification, primers were designed and synthesized to be used as primers for reverse transcription. The efficiency and specificity of reverse transcription was tested on ten samples by PCR and sequencing of the amplified fragments. As a result, the nucleotide sequence N of the rabies virus gene was obtained for ten samples.

The gene N encoding the nucleoprotein is known to be more conservative for all representatives of the genus Lyssavirus than the gene G encoding the envelope protein glycoprotein [9].

The obtained rabies virus isolates extracted from the territory of East Kazakhstan Region are genetically the closest to strains from Russia and China, thus it can be assumed that circulation of rabies virus to the eastern and central part of Kazakhstan from the Russian Federation and China was promoted by migration of wild carnivorous animals.

Thus, the first genotype - classical rabies virus (RABV) - circulates among domestic, agricultural and wild animals in the territory of East-Kazakhstan region.

Resulting from researches, in the territory of East Kazakhstan region the isolated strains of rabies virus were divided into two groups according to A.A. Devyatkin et al. [10], this is Arctic-like group, which includes genotypes from Primorski Krai and the most widespread group - Steppe group, which includes genotypes from Orenburg, Lipetsk, Novosibirsk, Astrakhan, Omsk regions, Altai Krai, Kazakhstan, Mongolia, PRC Inner Mongolia province and Xinjiang Uygur autonomous region.

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ШЫҒЫС ҚАЗАҚСТАН ОБЛЫСЫ АУМАҒЫНДАҒЫ ҚҰТЫРУ ВИРУСЫН МОЛЕКУЛЯРЛЫҚ-ГЕНЕТИКАЛЫҚ ТАЛДАУ

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Түйін

Мақалада генетикалық талдау мақсатында Шығыс Қазақстанның аумағында оқшауланған лиссавирусының штамдардың молекулярлық-генетикалық зерттеуі ұсынылған. Рабдовирусының нуклеопротеині бар тазартылған ПТР өнімдерінің он позитивті үлгісі зерттелді. Сонымен, филогенетикалық талдау нәтижесінде Шығыс Қазақстан облысындағы құтыру ауруының он толық нуклеопротеиндік тізбегі екі тобына жататын 6 типке жинақталды: арктикалық тектес топ және ең көп таралған топ - Степная.

Кілт сөздер: құтыру, ПТР, секвенирлеу, филогенетикалық ағаш, штамм, изолят, генотип.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ ВИРУСА БЕШЕНСТВА НА ТЕРРИТОРИИ ВОСТОЧНО-КАЗАХСТАНСКОЙ ОБЛАСТИ

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Аннотация

В данной статье представлены результаты секвенирования геномов изолятов лиссавирусов, выделенных от животных обитающих в Восточно-Казахстанской области с целью проведения генетического анализа. Происследовано десять положительных проб (ПЦР-продуктов), содержащих рабдовирус. В результате филогенетического анализа было собрано десять полных последовательностей нуклеопротеина вируса бешенства из Восточно-Казахстанской области в 6 генотипов, которые относятся к двум группам лиссавирусов: Арктически-подобная группа и наиболее распространенная группа – Степная.

Ключевые слова: бешенство, ПЦР, секвенирование, филогенетическое дерево, штамм, изолят, генотип.