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Polymorphism of the gene pol of the bovine leukemia virus

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As a representative of RNA-containing viruses, the causative agent of bovine leukemia (BLV) is characterized by the significant level of genetic variability which results in considerable information content of phylogenic studies of its populations. **Aim**. This study is aimed at exploring the sequence of 440 bp long fragment of the pol gene of BLV in three farms in the Eastern Ukraine. **Methods**. The polymerase chain reaction, sequencing and standard methods of isolating nucleic acids have been used. **Results**. The sequencing and the sequence analysis of the *pol* gene demonstrated the presence of three variants of sequences, corresponding to the isolates, identified in Kharkiv and Sumy regions and the sample of the proviral DNA from the FLK-BLV culture. **Conclusions**. The genetic homology of the sequences of the characterized isolates to the populations of viruses, circulating in the territory of Europe and the European part of the Russian Federation, has been established.

Key words: bovine leukemia virus, DNA, polymorphism, sequencing, phylogenic analysis.

INTRODUCTION

The viruses, containing RNA in their gene, are characterized by a high rate of the variability of nucleotide sequence and related considerable lability of the genetic material structure [1–3]. Similar to other RNA-containing viruses, the genomes of retroviruses are highly variable due to mutations, occurring in the process of matrix replication and possible genetic recombinations.

One of the representatives of retroviruses is the causative agent of BLV – bovine enzootic leukosis virus. The bovine enzootic leukosis virus is phylogenetically related to the viruses of the human T-lymphotropic virus type, HTLV-1 and HTLV-2, which explains a number of common clinical signs of disease, caused by these viruses. In particular, all of them are characterized by neoblastic processes, accompanying the immunosuppression and immunodeficiency state [4].

The genome of the causative agent contains both variable and conservative fragments. The hypervariable sites of the viral genome are genes *pol* and *tax*, the divergence of which is in the same range – up to 12−15 %. These genes encode the non-structural viral proteins. The study of their nucleotide structure gives the idea about the evolution of the causative agent and its origin.

As for Ukrainian isolates of BLV, previous studies comprised some molecular and epizootic investigations of the genetic structure of the sequences of gene *env*, which is the classical target during the genotyping of the causative agent.

The materials presented illustrate the data of the phylogenic analysis of three isolates of BLV, identified in the farms of the Eastern Ukraine, which are not BLV-free.

MATERIALS AND METHODS

The blood samples of cattle, stabilized with 5 % solution of sodium citrate, that had been taken from three farms in the Eastern Ukraine, which are not BLV-free, were used in the polymerase chain reaction (PCR) and the method of genome sequencing.

The method of affine sorption was used to extract the total DNA, which also contained probable BLV proviral DNA. The standard method, modified at NSC "IECVM" was used to isolate the nucleic acids.

The extracted DNA samples were used during the PCR to obtain variable fragments of the gene *pol* of

BLV. The BLV3 primer system, elaborated by NSC "IECVM", which is flanking the 440 bp long gene region, as well as the basic reagents for PCR (Fermentas, Lithuania) were used in the work. DNA-extracts obtained after the cultivation of FLK-BLV cell culture were used as a control sample.

The PCR products obtained were visualized in 1.5 % agarose gel after horizontal electrophoretic separation and staining with ethidium bromide. Specific monofragments were cut out of gel and extracted using Rapid Gel DNA extraction kit (Fermentas, Lithuania).

Chemical sequencing and DNA-analysis were conducted on the basis of SSI the State Center for Innovative Biotechnologies using the amplification equipment (Biorad, USA) and Beckman Coulter DNA-analyzer (Great Britain). DNA-analytes were obtained using GenomeLab™ DTCS Quick Start Kit (Beckman Coulter) for sequencing. The samples obtained were purified by precipitation and washed with ethanol. Then they were analyzed by their separation in the capillaries of the DNA-analyzer, filled with polyacrylamide gel.

The sequences (BLVNSC – BLV clone, NSC "IECVM"; BLVKH2 and BLVKH1 – isolate from Kharkiv Region; BLVSU – isolate from Sumy Region) were obtained and the sequences of the pol gene of virus isolates from the GenBank (AF257515, BLVCG, D00647, NC_001414 and AF033818) were transferred into FASTA format, with subsequent analysis using BioEdit (correction and multiple alignment) and MEGA 5.1 (neighbor-joining tree and its analysis).

RESULTS AND DISCUSSION

According to the amplification, the DNA fragments of specific length (440 bp) were identified in 4 out of 16 samples, obtained from farm 1 (Kharkiv Region), 8 out of 12 samples, obtained from farm 2 (Kharkiv Region), and 6 out of 10 samples – from farm 3 (Sumy Region). In addition, the fragment of specific length was formed in the course of studying the sample, extracted from FLK-BLV culture, which testifies to the success of the reaction.

After the corresponding correction and alignment there were four consensus sequences, 280–440 bp long, which, according to the results of on-line analysis using *blastn* algorithm in BLAST program, corresponded to the sequences of BLV matrices.

The analysis of sequences demonstrated that conventionally they form three variants: 1) isolates, identified in the territory of Kharkiv Region (indicated Kh1 and Kh2); 2) isolate from Sumy Region (Su), and 3) sample of proviral DNA from FLK-BLV culture.

Further phylogenic analysis involved obtaining the sequences of the gene *pol* of virus isolates from the GenBank nucleotide sequence database, identified in the territory of Europe (PL, RU, BE, IT, ES) and South America (BR). These were studied using the multiple

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	4805	4815	4825	4835	4845	4855
BLVNSC	TGGGTTCCCT	GGCGTTTGCTGG	AAAGCCTTCT	AAATGCCCAA	AGAACGACGG	TCTCGAAGAC
BLVKH2	TGGGTTCCCT	GGCGTTTGCTA	AAAGCCTTC	AAATGCCCAA	AGAACGACGG	TCTCGAAGAC
BLVSU	TG TGGAGC	GGCGATTAGAG	ATAACCCAAA	CATCCCCCGC	AAGACGGCGG	TCTCGAAGAC
BLVKH1	TG TCGAGT	CGTGCT GCTGT	ACANATTATT	CGGTGCTTAC	CTGTCCGGCGG	TCTCGTAGAC
AF257515	TGGGTTCCCT	GGCGTTTGTT	GAAAGCCTTC	AAATGCCCAA	AGAACGACGG	TCCCGAAGAC
BLVCG	TGGGTTCCCT	GGCGTTTGCT	GAAAGCCTTC	AAATGCCTAA	AGAACGACGG	TCCCGAAGAC
D00647	TGGGTTCCCT	GGCGTTTGCT	GAAGGCCTTC	AAATGCCTAA	AGAACGACGG	TCCCGAAGAC
NC_001414	TGGGTTCCCT	GGCGTTTGCT	AAAAGCCTTC	AAATGCCCAA	AGAACGACGG	TCCCGAAGAC
AF033818	TGGGTTCCCT	GGCGTTTGCT	AAAAGCCTTC	AAATGCCCAA	AGAACGACGG	TCCCGAAGAC

Fig. 1. Multiple alignment of the sequences of the gene *pol* of Ukrainian and European isolates of BLV: 8r – BLV clone IECVM; 5r – Kh2; 7r – Su; 3r – Kh1

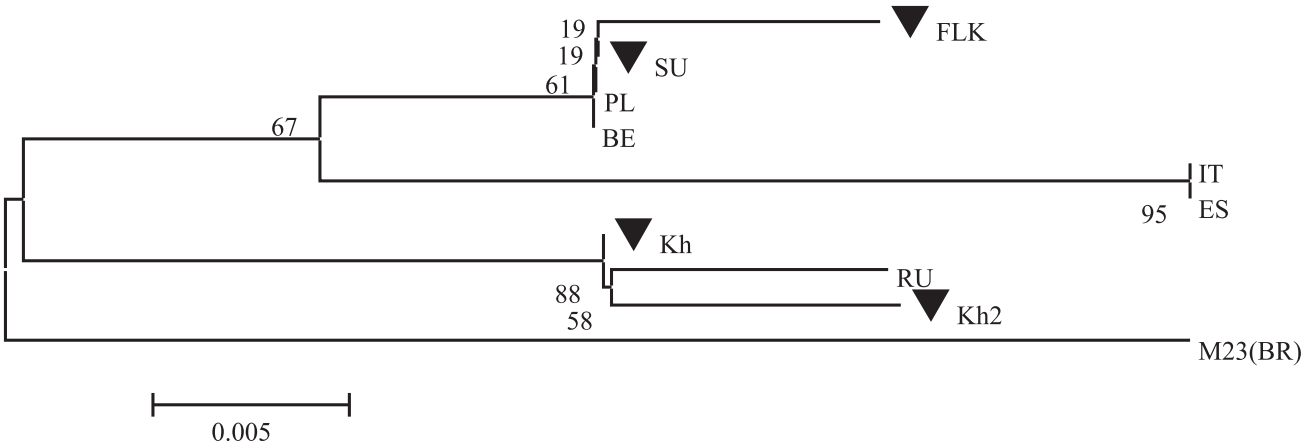

Fig. 2. The phylogenic relations of field BLV isolates according to the sequences of the gene *pol* (▼− identification mark of the Ukrainian isolates)

alignment method, which allowed identifying several insertions in the sequences of the gene *pol* (Fig. 1).

The repetition indices, demonstrating the share of topology repetitions during the multiple analysis of the calculated phylogenetic trees, were used as a criterion for the reliability of the dendrogram of the obtained topologies.

The dendrogram demonstrates the presence of two main clusters in the viral population according to the phylogenic distribution of the sequences of the gene *pol* (Fig. 2). The first one comprises the Russian isolate of BLV which is a typical representative of the subpopulation of the causative agent, which circulates in the European part of the Russian Federation. The isolates, identified in the farms of Kharkiv Region, demonstrated the identity to the abovementioned isolate. The divergence amid the group was less than 1% (Fig. 2).

The second branch proved to be more variable, as it contained two clades: South-European isolates and the isolates from the Central Europe. The divergence amid the clades was 0–0.6 % and between the groups – up to 4%. Isolate Su, which is a part of this group, was phylogenetically the closest to the causative agents from Poland and Belgium (the level of nucleotide differences < 0.01 %).

The sequence of the gene *pol* from the culture of FLK BLV cells had 0.7% divergence relative to these isolates.

CONCLUSIONS

The phylogenic analysis of the gene *pol* of the bovine enzootic leukosis virus isolates revealed their similarity to the Russian strains of the virus, the divergence between the sequences of the analyzed gene was from 0.01 to 0.8 %. The studies will be continued with the purpose of extending the spectrum of the analyzed isolates and involving the sequences of other BLV genes in the comparison.

Полиморфизм гена pol вируса лейкоза крупного рогатого скота

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Возбудитель лейкоза крупного рогатого скота (КРС) как представитель РНК-содержащих вирусов характеризуется существенным уровнем генетической вариабельности, что обусловливает значительную информативность филогенетических исследований его популяций. **Цель.** Изучить последовательности гена *pol* вируса лейкоза КРС из трех хозяйств Восточного региона Украины длиной 440 п. н. **Методы**. Использованы полимеразная цепная реакция, секвенирование и стандартные методы выделения нуклеиновых кислот. **Результаты.** Проведенное секвенирование и анализ последовательностей гена *pol* продемонстрировало наличие трех вариантов последовательностей, соответствующих изолятам, выявленным на территории Харьковской, Сумской областей, и образцу провирусной ДНК из культуры клеток FLK-BLV. **Выводы.** Установлено генетическую гомологию последовательностей охарактеризованных изолятов с популяциями вирусов, цирку-

лирующих на территории Европы и европейской части Российской Федерации.

Ключевые слова: вирус лейкоза КРС, ДНК, полиморфизм, секвенирование, филогенетический анализ.

Поліморфізм гена pol вірусу лейкозу великої рогатої худоби

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Збудник лейкозу великої рогатої худоби (ВРХ) як представник РНК-вмісних вірусів має істотні рівні генетичної варіабельності, що зумовлює значну інформативність філогенетичних досліджень його популяцій. **Мета.** Вивчити послідовності гена pol вірусу лейкозу ВРХ з трьох господарств Східного регіону України довжиною 440 п. н. **Методи.** Використано полімеразну ланцюгову реакцію, секвенування та стандартні методи виділення нуклеїнових кислот. **Результати.** Проведене секвенування та аналіз послідовностей гена *pol* продемонстрував наявність трьох варіантів послідовностей, що відповідають ізолятам, виявленим на території Харківської, Сумської областей, і зразка провірусної ДНК з культури FLK-BLV. **Висновки.** Встановлено генетичну гомологію послідовностей охарактеризованих ізолятів з популяціями вірусів, які циркулюють на території Європи та європейської частини Російської Федерації.

Ключові слова: вірус лейкозу ВРХ, ДНК, поліморфізм, секвенування, філогенетичний аналіз.

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