

Part 1. Veterinary Medicine

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STUDYING OF PHYLOGENETIC RELATIONSHIPS OF LEUKEMIA VIRUS WITH OTHER RETROVIRUSES IN CATTLE

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Summary. Bovine leukemia virus (BLV) is one of the retroviruses, which genetically, structurally and functionally related with viruses of human T-cell leukemia. BLV is very convenient model for studying the pathogenesis of humans leukemia. Genomes of retroviruses is high variability due to lack of a mechanism of correct errors that occur when copying matrix during replication, and possible genetic recombination. In this regard, the study of the genetic variability of the virus is one of the major objective for biological monitoring. At this time molecular genetic analysis (polymerase chain reaction (PCR) is a necessary part of phylogenetic research. The aim of this work was to study the variability of the bovine leukemia virus, establishing phylogenetic relationships between isolates sequenced bovine leukemia virus, which circulates in farms of different regions in Ukraine, with other animals retroviruses. Was conducted the sampling of clinical material from cattle farms from different geographical regions in Ukraine and extracted proviral BLV DNA. Total received 831 samples of peripheral blood from cattle farms in Kharkiv region, 10 samples — Kirovohrad region; 10 samples — Donetsk region; 41 sample — Crimea, Simferopol region; 10 samples — Poltava region. Sequenced gene fragments of *env* sequences of bovine leukemia virus proviral DNA, circulating in different geographical regions in Ukraine. Established isolates of bovine leukemia virus, circulating in Ukraine, belonging to the Euro-Asian subtype. Proved genetic affinity of leukemia virus and bovine syncytial virus, Jembrana disease virus and bovine immunodeficiency virus.

Keywords: DNA, phylogeographic relationships, polymerase chain reaction, sequencing, retroviruses, virus bovine leukemia.

Introduction. Bovine leukemia virus (BLV) is one of the retroviruses representative. Humans T-cell leukemia viruses, HTLV-1 and HTLV-2, genetically, structurally and functionally related to bovine leukemia virus, and the development of diseases caused by these viruses is similar (Willems et al., 1993; Dube et al., 1997). That is why BLV is a very suitable model for studying the pathogenesis of human leukemia. An important aspect of these studies is the problem of bovine leukemia virus genetic variability.

It is known that viruses, which genome is represented by RNA, characterized by high speed variation of nucleotide sequence and associated with it significant lability structure of genetic material (Steinhauer and Holland, 1987; Parvin et al., 1986; Steinhauer et al., 1989; Darlix and Spahr, 1983; Katz and Skalka, 1990; Meyerhans et al., 1989; Manini, De Palma and Mutti, 2007). Genomes of retroviruses, like other RNA-containing viruses, have high level of variability due to lack of mechanism for correcting errors arising until copying the matrix during replication and potential genetic recombination. Nucleotide modifications can lead to changes an amino acid consist of synthesized

proteins (Katz and Skalka, 1990). In this regard, the study of genetic variability of infectious agents is one of the main objectives of biological monitoring, that goal is the explanation of the phenomenon.

At this time, the necessary part of phylogenetic study is molecular genetic analysis (such as polymerase chain reaction (PCR) (Licursi et al., 2003; Giammarioli et al., 2008). The method of sequencing allows performing establish the existence of point and tandem mutations (Milos, 2009). Effectiveness and objectivity of molecular phylogenetic studies depends on many factors, such as: insufficient set of experimental data, errors in sequencing or sequence alignment, convergent evolution (i.e. formation of a complex of similar features in representatives from unrelated groups), horizontal gene transfer, etc. (Wendel and Doyle, 1998). In addition, different fragments of the genome provide unequal information during the molecular phylogenetic studies, because the result is more determined by the correct choice of gene or combination of genes in the sequence. Sometimes complementary DNA (cDNA), that includes coding sequences of structural genes, used for sequencing (Caraguel et al., 2009; Chang et al., 2009).

The aim of this work was to study the variability of the bovine leukemia virus, to establish phylogenetic relationships of sequenced isolates bovine leukemia virus that circulates in farms of different regions in Ukraine with other animals retroviruses.

Materials and methods. The total DNA was extracted from peripheral blood using a commercial kit 'DNA Sorbo-B' (Russia). Detection of proviral DNA was performed by PCR using basic kits Gene Pak™ (Russia) and a pair of primers III-BLV F/R developed in 2008. The length of the amplicon is 440 bp. Sequencing of proviral DNA of *env* gene fragments was performed on an automatic sequencer ABI PRISM 311D using the technology of ABI ('Applied Biosystems', USA).

Computer analysis of the primary structure of proviral DNA fragments of bovine leukemia virus isolates, multiple alignment of proviral DNA sequenced of major genes of retroviruses was carried out using programs Bioedit v. 7.0.0 (ClustalW modules and Neighbor) and Oligo Explorer v. 1.1.0. To construct phylogenetic trees used the program MEGA v. 4.1, and to view them — TreeView v. 1.6.6.

Results. There were sampling of clinical material of

cattle from farms of different geographical regions in Ukraine and proviral DNA of BLV was extracted. There were obtained 831 samples of peripheral blood of cattle from Kharkiv region farms, 10 samples — Kirovohrad region; 10 samples — Donetsk region; 41 samples — Crimea, Simferopol region; 10 samples — Poltava region.

To set the primary structure of fragments of BLV genomic material by sequencing, the samples of proviral DNA were transferred to the National Veterinary Research Institute (Pulawy, Poland). To determine the possible divergence and the genetic variability of bovine leukemia virus was conducted a multiple alignment and comparison of BLV *env* gene sequences circulating in the Kharkiv region and other geographical regions. For this purpose from the international database GenBank we have selected three fully sequenced BLV *env* gene sequences from Belgium (AF503581), USA (AY078387) and Brazil (AF399704) with length 1,548 bp, and partially sequenced fragment with length 960 bp from Poland (AF111171). Fragment of the multiple alignment of selected sequences shown in Fig. 1 (nucleotides that are not the same in this position with other nucleotides of selected sequences are underlined).

	425	435	445	455	465	475
AF111171	GGCCGATCAA	GGGTCCTTTT	ATGTCAATCA	TCAGATTTTA	TTCCCTGCATC	TCAAACAATG
AF399704	GGCCGATCAA	GGATCCTTTT	ATGTGCGATCA	TCAGATTTTA	TTCCCTGCATC	TCAAACAATG
AF503581	GGCCGATCAA	GGGTCCTTTT	ATGTCAATCA	TCAGATTTTA	TTCCCTGCATC	TCAAACAATG
AY078387	GGCCGATCAA	GGATCCTTTT	ATGTCAATCA	TCAGATTTTA	TTCCCTGCATC	TCAAACAATG
Kharkiv	GGCCGATCAG	GGATCCTTTT	ATGTCAATCA	TCAGATTTTA	TTCCCTGCATC	TCAAACAATG

	485	495	505	515	525	535
AF111171	TCATGGAATT	TTCACCTCTAA	CCTGGGAAAT	ATGGGGATAT	GATCCCCTGA	TCACCTTTTC
AF399704	TCATGGAATT	TTCACCTTTAA	CCTGGGAAAT	ATGGGGATAT	GACCCCTGA	TCACCTTTTC
AF503581	TCATGGAATT	TTCACCTCTAA	CCTGGGAAAT	ATGGGGATAT	GATCCCCTGA	TCACCTTTTC
AY078387	TCATGGAATT	TTCACCTCTAA	CCTGGGAGAT	ATGGGGATAT	GATCCCCTGA	TCACCTTTTC
Kharkiv	TCATGGAATT	TTCACCTCTAA	CCTGGGAGAT	AAAGGGATAT	GATCCCCTGA	TCACCTTTTC

	545	555	565	575	585	595
AF111171	TTTACATAAA	ATCCCTGATC	CCCCTCAACC	CGACTTCCCT	CAGCTGAACA	GTGACTGGGT
AF399704	TTTACATAAG	ATCCCTGATC	CCCCTCAACC	CGACTTCCCT	CAGCTGAACA	GTGACTGGGT
AF503581	TTTACATAAA	ATCCCTGATC	CCCCTCAACC	CGACTTCCCT	CAGCTGAACA	GTGACTGGGT
AY078387	TTTACATAAG	ATCCCTGATC	CCCCTCAACC	CGACTTCCCT	CAGTTGAACA	GTGACTGGGT
Kharkiv	TTTACATAAG	ATCCCTGATC	CCCCTCAACC	TGACTTACC	CAGTTGAACA	GTGACTGGGT

	605	615	625	635	645	655
AF111171	TCCCCTGTGTC	AGGTCATGGG	CCTGCTTTT	AAATCAAACG	GCACGGGCCT	TCCCAGACTG
AF399704	TCCCCTGTGTC	AGATCATGGG	CCTGCTTTT	AAATCAGACG	GCACGGGCCT	TCCCAGACTG
AF503581	TCCCCTGTGTC	AGGTCATGGG	CCTGCTTTT	AAATCAAACG	GCACGGGCCT	TCCCAGACTG
AY078387	TCCCCTGTGTC	AGATCATGGG	CCTGCTTTT	AAACCAAACA	GCACGGGCCT	TCCCAGACTG
Kharkiv	TCCCCTGTGTC	AGATCATGGG	CCTGCTTTT	AAATCAAACA	GCACGGGCCT	TCCCAGACTG

	665	675	685	695	705	715
AF111171	TGCTATATGT	TGGGAACCTT	CCCCTCCCTG	GGCTCCCGAA	ATATTAGTAT	ATAACAAAAC
AF399704	TGCTATATGT	TGGGAACCTT	CCCCTCCCTG	GGCTCCCGAA	ATATTAGTAT	ATAACAAAAC
AF503581	TGCTATATGT	TGGGAACCTT	CCCCTCCCTG	GGCTCCCGAA	ATATTAGTAT	ATAACAAAAC
AY078387	TGCTATATGT	TGGGAACCTT	CCCCTCCCTG	GGCTCCCGAA	ATATTAGTAT	ATAACAAAAC
Kharkiv	TGCTATATGT	TGGGAACCTT	CCCCTCCCTG	GGCTCCCGAA	ATATTAGTAT	ATAACAAAAC

	725	735	745	755	765	775
AF111171	CATCTCCAAC	TCTGGACCCG	GTCTCGCCCT	CCCGGACGCC	CAAATCTTCT	GGGTCAACAC
AF399704	CATCTCCAGC	TCTGCACCCG	GCCTCGCCCT	CCCGGACGCC	CAGATCTTCT	GGGTCAACAC
AF503581	CATCTCCAAC	TCTGGACCCG	GTCTCGCCCT	CCCGGACGCC	CAAATCTTCT	GGGTCAACAC
AY078387	CATCTCCAGC	TCTGGACCCG	GCCTCGCCCT	CCCGGACGCC	CAAATCTTCT	GGGTCAACAC
Kharkiv	CATCTCCAGC	TCTGGACCCG	GCCTCGCCCT	CCCGGACGCC	CAAATCTTCT	GGGTCAACAC

Figure 1. Fragment of multiple sequence alignment of *env* gene of bovine leukemia virus circulating in different geographical regions

The same value of divergence of the BLV *env* gene, circulating in the Kharkiv region, characterized proviral DNA sequences circulating in western Europe (Table 1). The smallest divergence observed for the *env* gene of

European isolates of bovine leukemia virus. In general, the results supported that the BLV *env* gene is highly conserved, and its primary structure does not change depending on the habitat of the causative agent.

Table 1 — The degree of similarity and divergence of the *env* gene of BLV circulating in different geographic regions, calculated relative to the corresponding sequence from isolate fragments of Kharkiv region

	AF111171 (Poland)	AF399704 (Brazil)	AF503581 (Belgium)	AY078387 (USA)
The number of nucleotides that do not coincide	14	15	14	19
Divergence, %	1.8	2.0	1.8	2.5
The degree of similarity, %	98.2	98.0	98.2	97.5

To establish phylogenetic relations between isolates of bovine leukemia virus circulating in Ukraine, and their phylogenetic relationships with isolates of other regions of the world (Europe, Asia, North and South America), was constructed the phylogenetic tree, that illustrated proximity of BLV isolates circulating in Ukraine to isolates of European and Asian subgroups (Fig. 2, 1).

BLV isolates, that proviral DNA was extracted from peripheral blood of animals from farms in Rivno, Poltava and Kharkiv regions, are closer to the European subgroup (Austria isolate). Bovine leukemia virus, circulating in the farms of Crimea, is closer to Asian subgroup (Zanjan, Tehran isolates). Isolates from the America form a separate, American, subgroup (Fig. 2, 2).

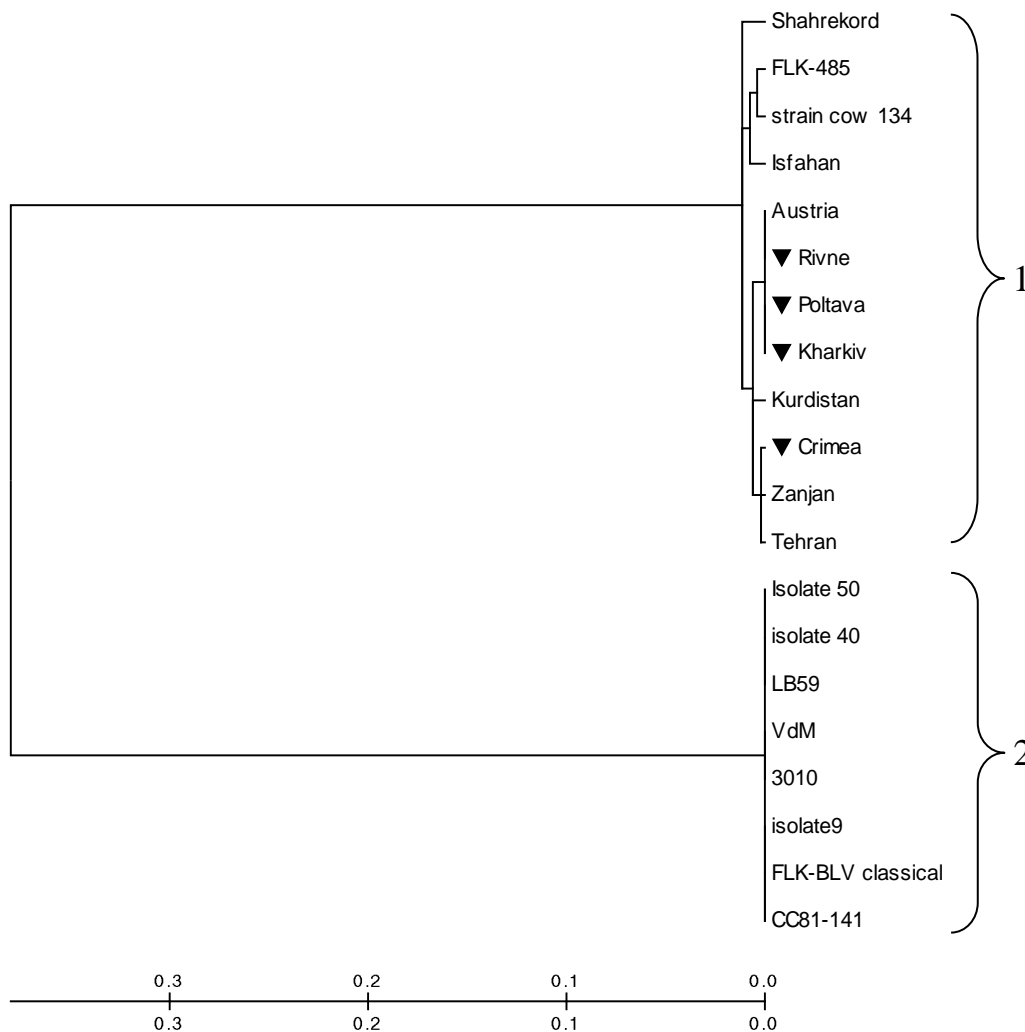


Figure 2. Dendrogram, based on *env* gene fragment of proviral DNA of bovine leukemia virus isolates from different geographical regions

Thus, the results of phylogenetic studies can be used to identify and study possible subgroups (or genotypes), to create the basis for the genes search, that determine the high biological activity of viruses.

To study the phylogenetic relationships of bovine leukemia virus was created databases of sequenced gene sequences and their fragments, isolated in different geographical regions and represented in international databases GenBank and EMBL: the bovine immunodeficiency virus, the Jembrana disease virus — lentivirus, that causes severe acute disease of cattle

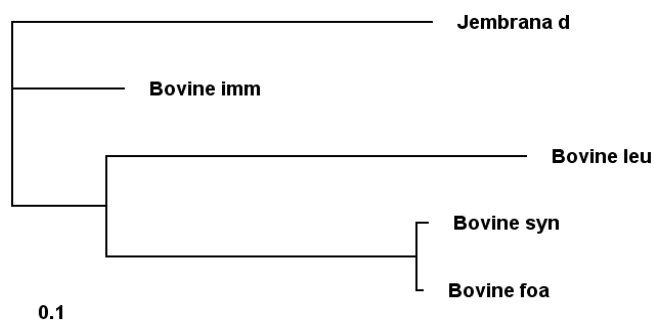


Figure 3. Dendrogram, based on *env* gene fragment of proviral DNA of bovine retroviruses isolates

Conclusions. Established that the BLV *env* gene is highly conserved, its primary structure has significant changes depending on the habitat of the causative agent. The greatest degree of similarity observed for *env* gene sequences of European BLV isolates. It was shown, that bovine leukemia virus isolates, circulating in farms of

characterized by lymphopenia and lymphadenopathy; syncytial virus; bovine leukemia virus.

Phylogenetic analysis, based on retroviruses *env* gene sequences (Fig. 3) and completely sequenced proviral DNA sequences (Fig. 4), showed firstly that isolates of Jembrana disease viruses, immunodeficiency and syncytial viruses form separate branches; secondly, membership of bovine leukemia virus isolates to one cluster; thirdly, the evolutionary closeness of the leukemia virus and bovine syncytial virus.

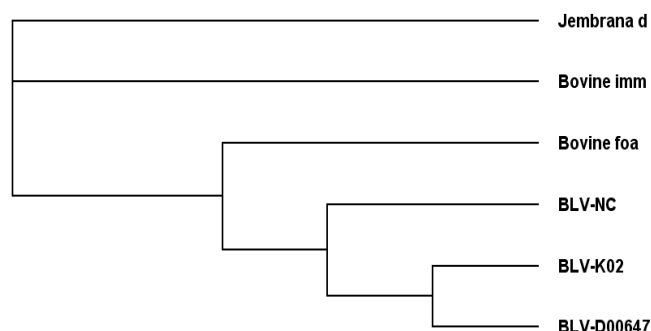


Figure 4. Dendrogram, based on complete sequences of proviral DNA of bovine retroviruses isolates

different geographic regions in Ukraine, are closer to the Euro-Asian subgroup. Based on phylogenetic analysis, proved the genetic proximity of leukemia virus and bovine syncytial virus, immunodeficiency virus and Jembrana diseases virus.

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