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).

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                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   425        435        445        455        465        475
AF111171        GGCCGATCAA GGGTCCTTTT ATGTCAATCA TCAGATTTTA TTCCTGCATC TCAAACAATG
AF399704        GGCCGATCAA GGATCCTTTT ATGTCGATCA TCAGATTTTA TTCCTGCATC TCAAACAATG
AF503581        GGCCGATCAA GGGTCCTTTT ATGTCAATCA TCAGATTTTA TTCCTGCATC TCAAACAATG
AY078387        GGCCGATCAA GGATCCTTTT ATGTCAATCA TCAGATTTTA TTCCTGCATC TTAAACAATG
Kharkiv         GGCCGATCAG GGATCCTTTT ATGTCAATCA TCAGATTTTA TTCCTGCATC TCAAACAATG
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   485        495        505        515        525        535
AF111171        TCATGGAATT TTCACTCTAA CCTGGGAAAT ATGGGGATAT GATCCCCTGA TCACCTTTTC
AF399704        TCATGGAATT TTCACTTTAA CCTGGGAAAT ATGGGGATAT GACCCCCTGA TCACCTTTTC
AF503581        TCATGGAATT TTCACTCTAA CCTGGGAAAT ATGGGGATAT GATCCCCTGA TCACCTTTTC
AY078387        TCATGGAATT TTCACTCTAA CCTGGGAGAT ATGGGGATAT GATCCCCTGA TCACCTTTTC
Kharkiv         TCATGGAATT TTCACTCTAA 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 CGACTTTCCC CAGTTGAACA GTGACTGGGT
Kharkiv         TTTACATAAG ATCCCTGATC CCCTTCAACC TGACTTACCC CAGTGGAACA GTGACTGGGT
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                   605        615        625        635        645        655
AF111171        TCCCTCTGTC AGGTCATGGG CCCTGCTTTT AAATCAAACG GCACGGGCCT TCCCAGACTG
AF399704        TCCCTCTGTC AGATCATGGG CCCTGCTTTT AAATCAGACG GCACGGGCCT TCCCAGACTG
AF503581        TCCCTCTGTC AGGTCATGGG CCCTGCTTTT AAATCAAACG GCACGGGCCT TCCCAGACTG
AY078387        TCCCTCTGTC AGATCATGGG CCCTGCTTTT AAACCAAACA GCACGGGCCT TCCCAGACTG
Kharkiv         TCCCTCTGTC AGATCATGGG CCCTGCTTTT AAATTCAAACA 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
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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*).

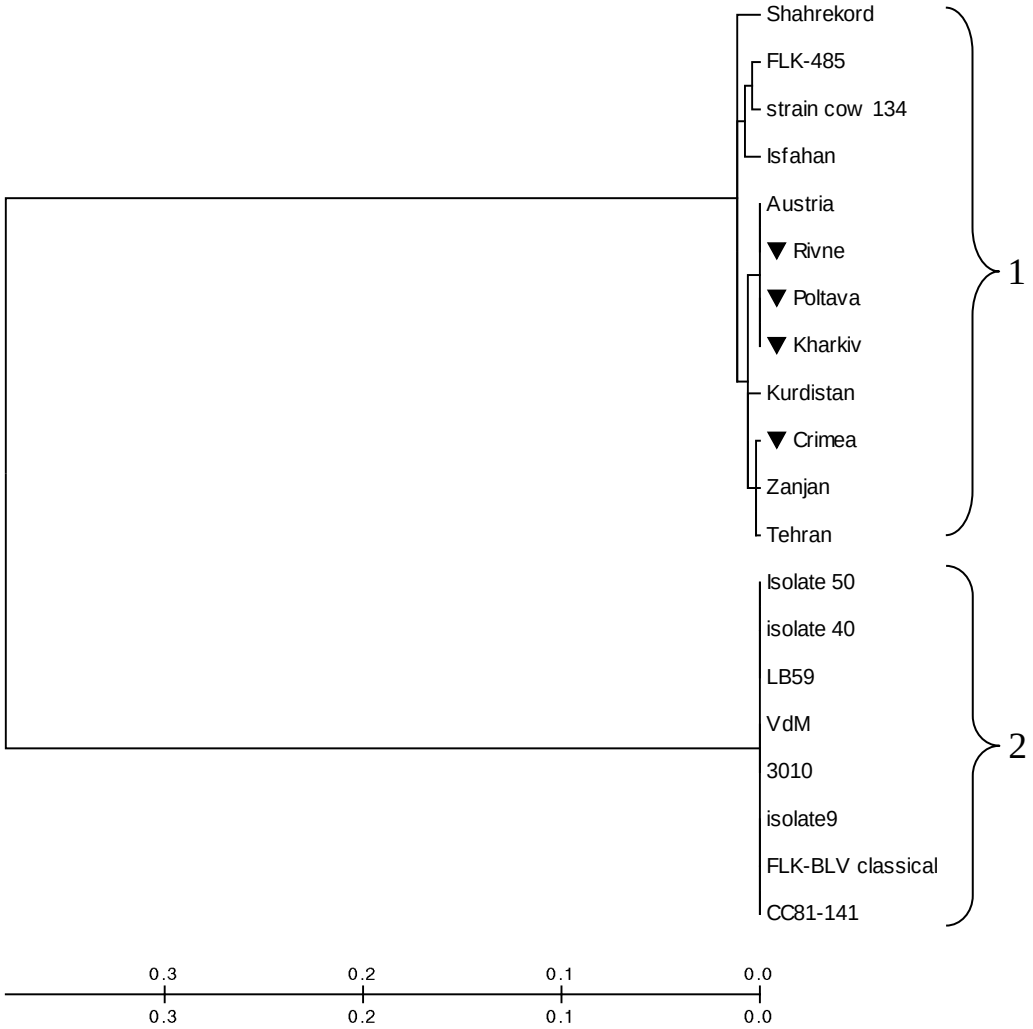

Figure 2. Dendrogram, based on *env* gene fragment of proviral DNA of bovine leukemia virus isolates from different geographical regions

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 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.

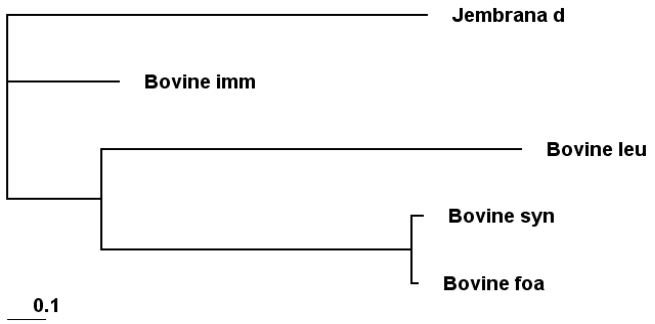

Figure 3. Dendrogram, based on *env* gene fragment of proviral DNA of bovine retroviruses isolates

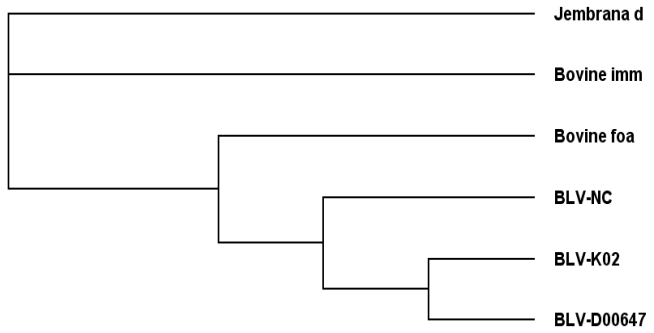

Figure 4. Dendrogram, based on complete sequences of proviral DNA of bovine retroviruses isolates

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 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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