

Revisiting the Issue of the Molecular-genetic Structure of the Causative Agent of the Bovine Leukemia Virus in the Russian Federation

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Abstract

Objectives: The study is aimed at investigating the genotypic diversity of the causative agent of Bovine Leukemia, circulating in animal populations from different regions of Russia. **Methods:** Genotyping and sequencing for samples of 444 bp gene DNA element of cattle HVL isolates of Russian regions were conducted and phylogenetic analysis of the isolated strains was performed. **Findings:** Blood samples, serum and leukoconcentrate of animal blood and DNA samples – env gene region, 444bp long, of cattle leukemia virus were a subject of investigation. Samples from Belgian subgroup (type B) and Australian subgroups (type A) of leukemia virus were singled out in the studied samples. The results of this study provide an opportunity to study features of leukemic epizootic process in the region. For the first time in the middle Urals in Russia the molecular-genetic structure of the cattle leukemia virus was singled out. Phylogenetic analysis of virus derived from DNA sequencing has allowed classifying the studied strains and comparing them with global isolates. Determination of the virus genotype allows for deeper research to identify the pathogenetic variants of leukemic process development in a particular region – in the middle Urals. Study of genotypic diversity of the cattle leukemia virus opens new opportunities and additional approaches to the development of methods for health improvement in leukemia disadvantaged populations. **Applications/Improvements:** The data obtained will serve as a base for the creation of highly sensitive, specific primers for effective PCR diagnostics.

Keywords: Cattle, Chain Reaction Method, DNA-sequencing, Genotype, Leukemia Virus, Molecular-genetic Structure, Polymerase

1. Introduction

Nowadays, especially during the introduction of retaliatory sanctions on livestock products, imported from the (EU) European Union to the Russian Federation, the demand for qualitative, competitive food products produced in the Russian Federation has increased. Its infection

safety is one of the most important competitive power parameters in accordance with the rules and regulations of the Customs Regulation. In this regard, the demand for fundamental and innovative approaches to the diagnosis and prevention of infectious animal diseases, aiding the most rapid health improvement of the cattle population in the Russian Federation, has increased sharply.

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Bovine Leukemia is a malignant lymphoproliferative virus disease. Retrovirus of Bovine Leukemia Virus (BLV), referring to *Retroviridae* family, *Deltaretrovirus* genus, integrates into the DNA of the host leukocytes and remains dormant in a great number of cells for a long period of time, which complicates its identification and detection of infected animals. The described disease refers to the most common chronic infectious diseases of farm animals in many countries all over the world, including Russia. Over the past 15 years, in the Russian Federation the BLV infection rate has not changed and ranges within the limits of 10.3 – 14%, and Leukemia share in the structure of infectious diseases of cattle exceeds 50%.

The disease is widespread around the world, and almost all countries take measures to combat Leukemia. Some US farms were troubled by Leukemia, but their products are intended only for internal use. In the EU countries, the work on the herd health improvement against this disease was launched in the 1970-1980s. Nowadays, the EU countries are safe in terms of Leukemia. They provide Mandatory Leukemia Certification of all livestock.

The safety criteria are very strict: the certificate is issued only in case of at least two-year absence of any manifestations of the disease (in laboratory or clinical studies).

In the United States, the program, which led to recovery of the majority of large and small enterprises in the country, was adopted in the 1980s.

In 2010, the Customs Union Regulations, which specify that the raw milk and dairy products, meat and meat products must be produced by the enterprises which are Leukemia safe during the last 12 months, came into force.

Especially it should be noted that Belarus, a member of the Customs Union, is, in general, Leukemia safe for the past 5 years (the detection level of the positively reacting animals in laboratory studies does not exceed 0.03%). Therefore, the dairy products of agricultural enterprises of the Russian Federation, by virtue of presence of animals, infected by the Leukemia agent, will certainly be less competitive.

The special concern is caused by the degree of Leukemia affected cattle in breeding farms in the Russian Federation where 386 (25.5%) of 1,513 breeding enterprises, having the appropriate licenses, are not Leukemia safe. 30% of breeding enterprises are identified as those having animals, infected by Leukemia Virus, which is a base of the Leukemia infection spread in different regions of the country through young livestock marketing.

It was proven that the existing methods of Leukemia diagnosis are highly-sensitive and specific. They allow identifying virus carriers early in the course of the disease. With the right arrangement of the anti-Leukemia measures and the control of their full implementation, livestock enterprise can be improved in 3-5 years on average.

Successful implementation of health-improving programs in the Russian Federation is in place: this disease was completely extirpated in the Sverdlovsk Region with livestock of 130,000 cows, in both public and private livestock breeding; in the Leningrad Region – in public livestock breeding; in 2008, the number of unsafe objects was reduced by 4 times (from 186 to 76 in 2015) in the Tyumen Region.

Since 2004, 174 unsafe farms have been improved in the Krasnodar Region. Health-improving measures are more effective with scientific and methodological support of leading Leukemia researchers of the Russian Research Institute for Experimental Veterinary, Ural Scientific Research Veterinary Institute, Institute of Experimental Veterinary of Siberia, the Far East State Scientific Institution and others.

Various diagnostic approaches exist for the purpose of cattle blood sample testing for the BLV virus, particularly the serological (Immunodiffusion Test, Enzyme-Linked Immunosorbent Assay) and molecular genetic (Polymerase Chain Reaction) methods. However, it is impossible to identify the BLV early in the course of the infection by serological 'indirect' methods, which is especially important for timely isolation of healthy calves from the BLV-infected animals. Polymerase Chain Reaction is a more sensitive 'direct' method of the pathogen identification, but it is not always effective at low BLV proviral load early in the course of the disease.

Currently, many scientists around the world monitor studies of the molecular-genetic structure of the Bovine Leukemia Virus¹⁻⁶.

At the initial stage of infection, the BLV penetrates into the chromosomal DNA of lymphocytes in the form of provirus in small numbers of 1-3 copies per diploid genome^{7,8}, and the animal becomes a virus carrier for the whole life.

In the host organism, chronically persistent virus can undergo mutational changes, intended for increase of the pathogenicity, overcoming more effective host defense mechanisms. Therefore, along with the genetic status of the microorganism, it is necessary to consider the genetic

variability of the pathogen, which may, with the long-term persistence, be able to change its pathogenicity towards either the commensalism or strengthening of pathogenic properties^{9,10}.

The data on the functional significance of polymorphisms, pathogenic properties of the genotypes, their ability to avoid immune surveillance by the host are not fully studied¹⁰.

It is indicated in ⁴ that the genetic variants of BLV with certain amino acid substitutions in gp51 protein epitopes were found in 7.5% of the animals being infected by the BLV, but still seronegative. Such variants can avoid detection of antibodies and will significantly interfere with the serological diagnosis of BLV infection.

During the infection, the majority of BLV-infected animals stays clinically healthy, but one third of them suffer from persistent lymphocytes, resulting from the polyclonal proliferation of B-lymphocytes, primarily, CD5 + cells, and only 0.1-10% of the animals suffer from lymphoid tumors, as it was observed.

Like other complex retroviruses, BLV genome contains structural and regulatory genes – gag, pol and env genes. The env gene encodes the gp51 and gp30 transmembrane glycoproteins of virus capsid, which cause the virus infectivity and detection of neutralizing antibody response during the immune response.

Genetic changes within the *Deltaretrovirus* genus are minimal as compared to lentiviruses of humans and animals. Full comparison of the genomes of the Belgian, Japanese and Australian BLV isolates identified high nucleotide homology (97%). Analysis of genetic variation of gp 51 sequences also showed a significant number of conservative BLV isolates in several geographic locations. These studies are consistent with the ones, previously conducted by ¹¹ and ⁴ who showed only minor differences, mostly, point (single base) mutations in the range of seven to twenty-two geographically different strains, respectively. These mutations were characterized by some differences in restriction sites, enabling to categorize BLV isolates into three and six genotypes according to the RFLP analysis. It was also shown that some genotypes may affect the leukemia virus genesis. In ¹² reported the connection between some BLV variants and the inability to detect antibodies in the infected cattle, but ¹³ and ¹⁴ observed no correlation between individual genotypes and serological status of infected animals.

Characterization of the global genetic diversity of

BLV is the current task of international research efforts. Previous studies of phylogenetic analysis found that the env sequences, received from other countries, can be grouped into three or four different genetic subgroups. Other studies, recently conducted by ¹⁵, which include the study of the available sequence with different geographical origins, clearly showed that the BLV isolates can be classified into seven different genotypes. However, this classification was based on the available data on sequences; basically it contains BLV strains, circulating in the cattle population of the United States and South America.

Currently, there are no comprehensive monitoring studies, dedicated to genetic characterization and classification of BLV isolates, found in Eastern Europe and Russia.

2. Purpose, Objectives and Methodology of the Study

The purpose of this study was to investigate the genotypic diversity of the causative agent of Bovine Leukemia, circulating in animal populations from different regions of Russia.

To achieve the purpose, the following objectives were set:

1. To conduct the genotyping and sequencing of the DNA region sample of the 444 bp gene of BLV isolates in Russian regions.
2. To perform Phylogenetic Analysis of isolated strains.

Experimental studies for DNA-sequencing of samples, taken from the blood of animals in the different regions of Russia, were conducted in the Department of Biochemistry of the Poland National Veterinary Research Institute (Pulawy), Director – Tadeusz Wijaszka, with involvement of Professor Jacek Kuzmak.

The following data were used for epizootological analysis: the statistical data of veterinary reports, the results of diagnostic studies on Leukemia by the veterinary laboratories in Sverdlovsk, Tyumen, Kurgan and Chelyabinsk regions, the results of studies of the biological material conducted in the Ural Scientific Research Veterinary Institute and National Research Veterinary Institute in Poland.

The object of the study was the bovine cattle of Ural

type of black-and-white cattle, belonging to agricultural enterprises in Urals Federal District.

The subject of the study was the blood sample, a blood serum and leukoconcentrate from the blood of animals and samples of DNA region of env gene 444bp long of bovine leukemia virus.

Laboratory diagnostic tests of blood, serum of animal blood with the help of Immunodiffusion Test, Enzyme-Linked Immunosorbent Assay and Polymerase Chain Reaction were carried out according to the Diagnosis of Bovine Leukemia Guidelines, approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation as of August 23, 2000.

Comparison of the efficacy of different laboratory methods for diagnosing BLV-infected animals was carried out in livestock enterprises of the Urals.

In the recovered herds of cattle, the blood samples of cows and heifers of various ages were taken. The blood samples obtained from experimentally infected sheep and rabbits were taken as a template. Then, the obtained material was tested by different methods at the same time: Immunodiffusion Test (IT), Enzyme-Linked Immunosorbent Assay (ELIA), Polymerase Chain Reaction (PCR) and Nested Polymerase Chain Reaction (PCR).

Blood samples for PCR studies were taken from animals and put into the Venosafe-Hematology VF-0539DK (manufacturing country – Belgium) vacuum tubes containing ethylenediaminetetraacetic acid. The study was carried out using the 'Leukemia' test systems, manufactured by Central Research Institute of Epidemiology of Rospotrebnadzor, Federal Budget Institution of Science (Moscow), according to the instructions approved by the Rosselkhoz nadzor on February 29, 2006, as well as primers, manufactured by the Russian Central Research Institute of the Fats and Oil Industry of the Russian Academy of Agricultural Sciences¹⁶.

The investigation of samples through ELISA was carried out using the Veri-test kit for detection of antibodies to BLV (manufactured by Narvak, Manufacturing and Research Association, Moscow) according to the instruction No. 13-7-2/2130 as of August 23, 2000, approved by the Veterinary Department of the Ministry of Agriculture of the Russian Federation, and the SERELISA® BLV Ab Mono Blocking kit (manufactured by Synbiotics, France).

The diagnostic kit, manufactured by BIOK (Kursk biological-manufacturing plant) was used for the Immunodiffusion Test (IT) diagnostics. The investigation was conducted according to instruction No. 13-7-2/2130 as of August 23, 2000, approved by the Veterinary Department of the Ministry of Agriculture of Russia.

Genomic DNA was isolated and purified from the PCR product (primers, nucleotides, polymerases, dNTP) by Nucleo Spin Extract® II set (MACHEREY-NAGEL GmbH & Co. KG), according to the method, described by foreign authors^{4,11–13,17–19}, and its concentration was evaluated by spectrophotometry, for which process the GeneQuantII DNA Calculator (Pharmacia Biotech) device was used. Molecular biology class water, free of nucleases, filtered by 0.22-micron membrane (manufactured by Fermentas), was used as H₂O.

Formation of primary and Nested PCR was performed according to the methods described by ^{4,18}.

Env-Nested PCR was performed with pair of primers: env₅₀₃₂ and env_{5608r} (external primers ending with the 608bp amplification of fragments) and env₅₀₉₉, env_{5521r} (internal primers ending with the 444bp amplification of fragments).

Oligonucleotide primers for PCR were designed according to the sequence data, published by ⁸ Primers corresponding to env gene were chosen because this area is common to different strains of BLV proviruses^{7,8,11}.

All primers used in this experiment were obtained from the MWG-Biotech (Ebersberg, Germany).

Amplification was performed according to the methods, described by ¹⁸. The amplification reaction was performed in 480 DNA thermal cycler (Perkin-Elmer Cetus, Inc., Weathersfield, Germany) or TRIO Thermoblock (Biometra, Uettingen, Germany).

Gene Ruler (1Kb DNA) Ladder Plus, Fermentas (0.1 mg/ml) was used as the marker, and Loading buffer, Fermentas was used as the loading buffer. The electrophoresis reaction was carried out using 1.5% agar gel after the staining with ethidium bromide and was visualized in the Gel Doc system Bio-Rad Laboratory (BIORAD).

The study of env 444bp gene polymorphism was performed according to the methodologies, described by the following authors.^{4,11,17–18}

Sequence of isolated PCR product during the sequencing of env 444bp gene site of BLV was determined using Terminator v.3.1 (Applied Biosystems – Life

Technologies), a set of fluorescent, coloring dideoxy terminator sequencing cycle, and analyzed in 3730xl DNA Analyzer (Applied Biosystems – Life Technologies). Phylogenetic tree of the nucleotide sequences of various BLV strains was drawn up with the help of MEGA 4 software package (molecular evolutionary genetics analysis, version 4.0)²⁰.

World strains, studied for comparison during the phylogenetic analysis, were taken from Gen Bank²¹.

Statistical processing of the digital data was performed using Microsoft Office 2003 standard programs on a personal computer.

3. Results of the Study and Discussion

All samples were taken from the positively responding (according to the latest investigations in IT) cows aged 3-4 years, hematologically sick animals and calves aged 3.5-4 months. The samples were selected from various agricultural enterprises of Koelga (1C, 7C, 8C, 9C, 10C), Etkulsk (Ural 1, Ural 2) districts of the Chelyabinsk Region, as well as from the Yalutorovsk district of the Tyumen Region (1s, 2s, 3s, 4s, 5s). Table 1 gives characteristics of the analyzed material.

PCR is an analysis focused on the identification of Leukemia provirus in cows' blood,³ it has shown that not

all IT-positive animals are virus carriers of the detected fragment of the env gene. DNA analysis of 30 cows from the Chelyabinsk Region found that 65.00% of IT-positive (Ural 1) and 90.0% of hematologically sick animals were the carriers of the Leukemia virus detected by PCR method. This fact may be connected with the reason that the animals are at a resistant alymphatic stage or at an early stage of persistent lymphocytosis, which conditions low levels of proviral DNA in the blood of cows. The chronically persistent virus can undergo the mutational changes, intended for the increase of the pathogenicity, overcoming of more effective host defense mechanisms²².

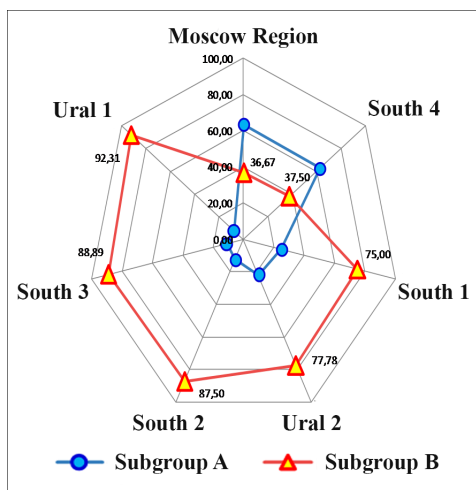
Until the end of 2010, BLV strains, singled out all over the world, were classified into seven different genotypes^{5,15}. Currently, at least eight evolutionary genotypes of Leukemia virus are singled out²³.

The performed PRC-RFLP (Restriction Fragment Length Polymorphism) analysis of the env gene fragment of BLV of 444 bp length showed that two genetic types of Leukemia virus, which are related to viruses of 'Australian' and 'Belgian' subgroups according to the International Classification, are circulated in the studied regional populations.

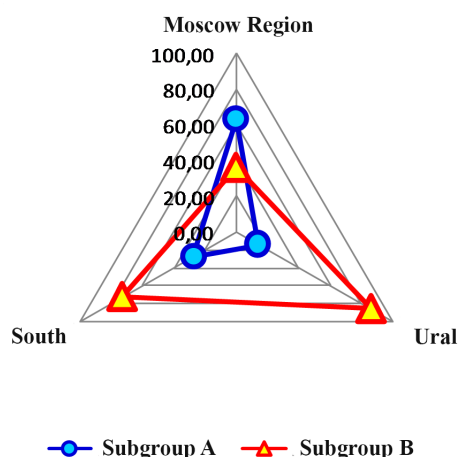
The studies have shown that two types of Leukemia virus, related to the viruses of 'Australian' and 'Belgian' types according to the genetic classification, are circulated in two farm units of the Chelyabinsk region (Figures 1A, 1B).

Table 1. Characteristics of the Analyzed Material, studied in the Russian Scientific-Research Institute of Livestock Breeding (State Scientific Institution) of the Russian Academy of Agricultural Sciences

Ser. No.	District	Breed	Livestock unit number, n	BLV Status		PCR+, %
				IT+	including hematology «+»	
1	Moscow Region	Black-and-White	30	30	8	100.00
2	Ural 1	Black-and-White	20	20	-	65.00
3	Ural 2	Black-and-White	10	10	10	90.00
4	South 1	Ayrshire	10	10	-	80.00
5	South 2	Holstein	10	10	-	80.00
6	South 3	Holstein	10	10	-	90.00
7	South 4	Ayrshire	10	10	-	80.00
Total:			100	100	18	85.00



(A)



(B)

Figure 1. Molecular Genetic Characteristics of the Virus Types in the Geographical Aspect.

Note:

Subgroup A: Australian type, showed with the help of circular marker; Subgroup B: Belgian type, showed with the help of triangle marker.

Analyzing the data of Figure 1A, one should note that the infection of Belgian type of virus in the farm units of the Chelyabinsk Region was at the level of 77.78% in the Ural 2 farm unit and up to 92.31% in the Ural 1 farm unit. The detected virus of animals of Ural 2 group, in which the hematologic stage of BLV were detected, refers mostly to the Belgian type, and the ratio was 1: 3.5 (Figure 2). Thus, the ability of both the Australian and Belgian types of the virus to cause hematologic stage of the disease was shown.

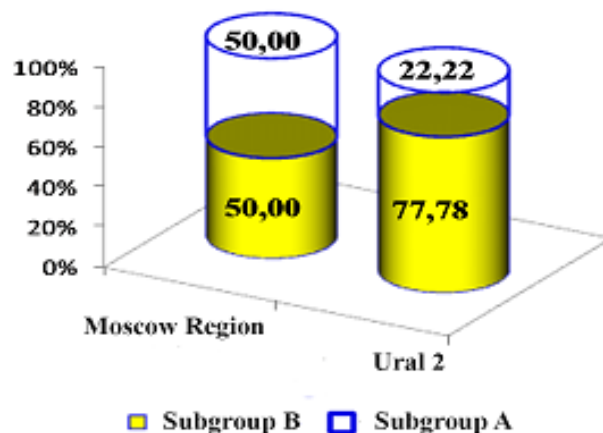


Figure 2. Distribution of BLV Genotypes in Case of the Hematological Stage of the Disease.

Note: animals, infected with the Australian type of BLV, are shown as the upper cylinder, and those, infected with the Belgian type of BLV, are shown as the bottom cylinder.

Phylogenetic studies of populations, carried out in the Russian Scientific-Research Institute of Livestock Breeding (State Scientific Institution) of the Russian Academy of Agricultural Sciences, based on the analysis of restriction polymorphisms of the env gene, have shown that the whole investigated animal population is grouped into two clusters (Figure 3).

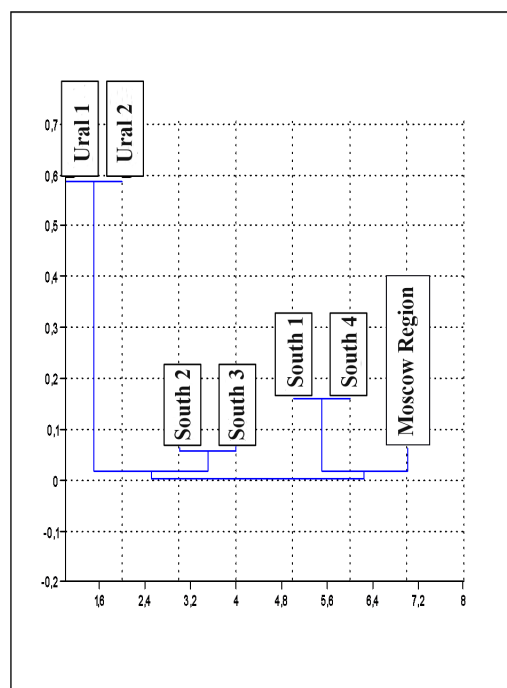


Figure 3. Phylogenetic analysis of populations.

The first cluster includes two farm units of the Ural region and two farm units of the South part of Russia – South 1 and South 3. The second cluster is formed by animals of two farm units – South 2 and South 3, which also include the animals from farm units of the Moscow Region. For the final results on geno-geographical phylogenetics of BLV, it is necessary to carry out the sequencing of the env gene of the selected region.

Not quite the same situation was observed in the DNA samples, studied by us in the Poland National Veterinary Research Institute using the RFLP and DNA – sequencing. The samples were taken from the RID (+) and hematologically sick animals of the Yalutorovsk district of the Tyumen Region and Koelga district of the Chelyabinsk Region (Figure 4).

The obtained data allow referring BLV isolates of the Chelyabinsk Region to the Belgian type B, since during the analysis of restriction fragments each of the enzymes reacted

typically for the B subgroup of provirus (Bam HI did not form fragments, BclI split it into sites 225 and 219 bp long, and PvuII split it into sites 280 and 164 bp long). DNA samples of imported cows from Yalutorovsk district of the Tyumen Region have shown different results in RFLP analysis. Thus, the Bam HI enzyme split all amplicons into fragments 128 and 316 pb long; BclI – into segments 225-219 bp long; PvuII in samples No. 1s, 2s, 4s did not form the fragments, which is typical for the Australian or Japanese subgroup, that is why these samples were referred to the Australian subgroup (in the analysis of each of the enzymes), and in samples No. 3s and 5s it formed fragments 280 and 164 bp long, which is typical for the Belgian subgroup provirus, so these samples with comprehensive analysis can be referred to the Australian or Belgian subgroup (A/B).

The summarized analysis data of the distribution of restriction fragments for each of the enzymes (Bam HI, Pvu II, BclI) is shown in Table 2.

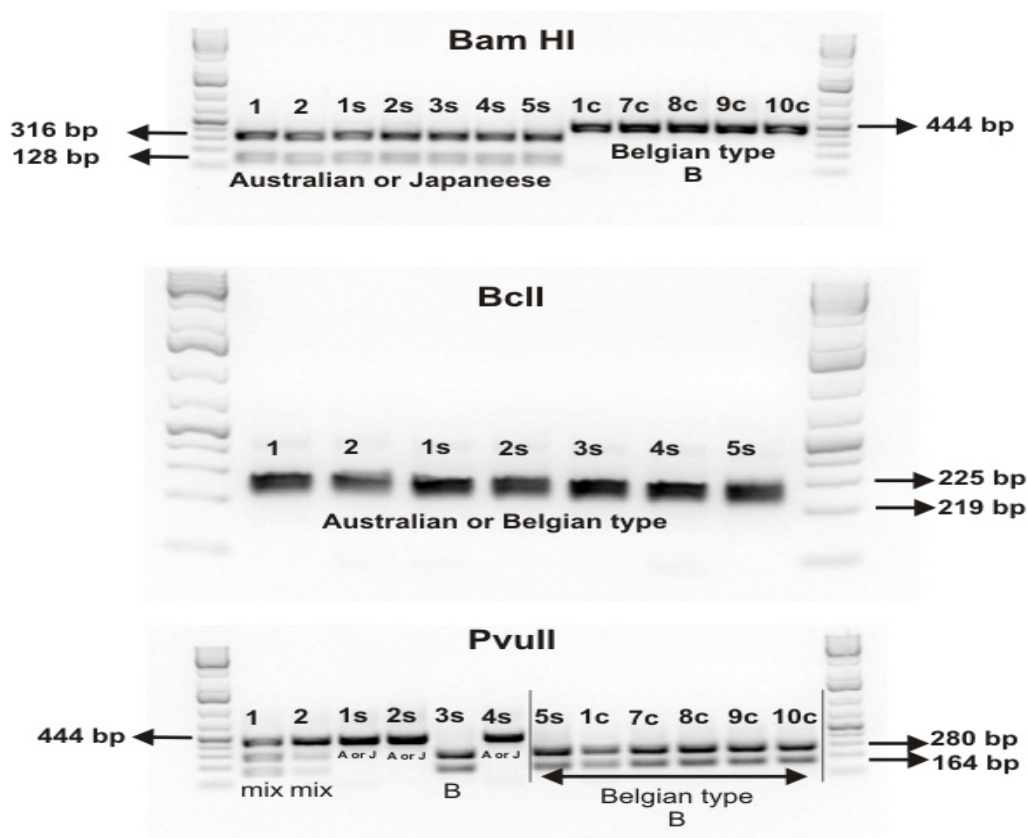


Figure 4. Electrophoregram of the distribution of restriction sites during the RFLP analysis of env gene of BLV with 3 restriction enzymes (BamHI, PvuII, and BclI) in test samples of DNA of animals from different regions of the urals.

Lines: M - molecular marker weighting 100 bp ; 1, 2 - DNA samples from the sheep; 1s, 2s, 3s, 4s, 5s - DNA samples from cows of Tyumen region; 1c, 7c, 8c, 9c, 10c - DNA samples from cows of the Chelyabinsk region.

Table 2. Results of the BLV genotyping from the isolates, obtained in the Chelyabinsk and Tyumen regions

№.	Region	Endonucleases and their restriction fragments	Classification			
			BanHI	BclI	PvuII	Beier et al., 2001
1S	Tyumen Region		315/129	226/218	444	Australian
2S			315/129	226/218	444	Australian
3S			315/129	226/218	281/163	Not classified
4S			315/129	226/218	444	Australian
5S			315/129	226/218	281/163	Not classified
1C	Chelyabinsk Region		444	226/218	281/163	Belgian
7C			444	226/218	281/163	Belgian
8C			444	226/218	281/163	Belgian
9C			444	226/218	281/163	Belgian
10C			444	226/218	281/163	Belgian

Genotyping researches of the env 444bp gene site defined the type of virus circulating in the farm units of the Tyumen and Chelyabinsk Regions.

The obtained data allow referring BLV isolates of the Chelyabinsk Region to the Belgian type B. In RFLP analysis a part of DNA isolates from cows imported from Yalutorovsk district of the Tyumen Region was referred to the Australian subgroup (in the analysis of each of the enzymes), and the part of such isolates formed fragments which are typical for both Belgian and Australian subgroups (A/B), see Figure 5.

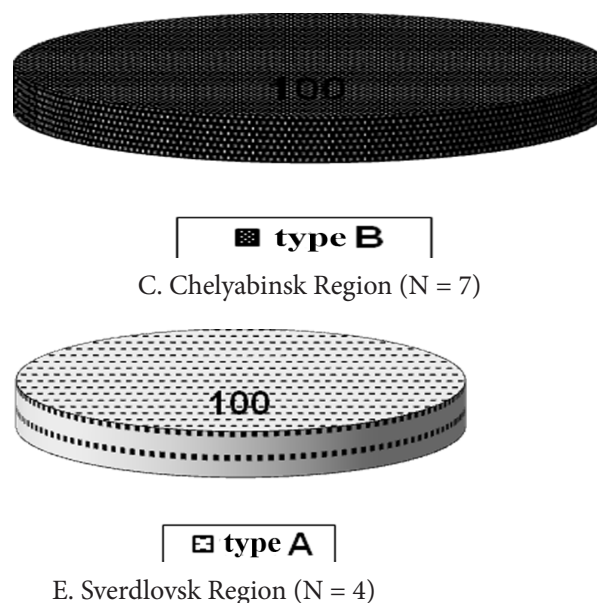
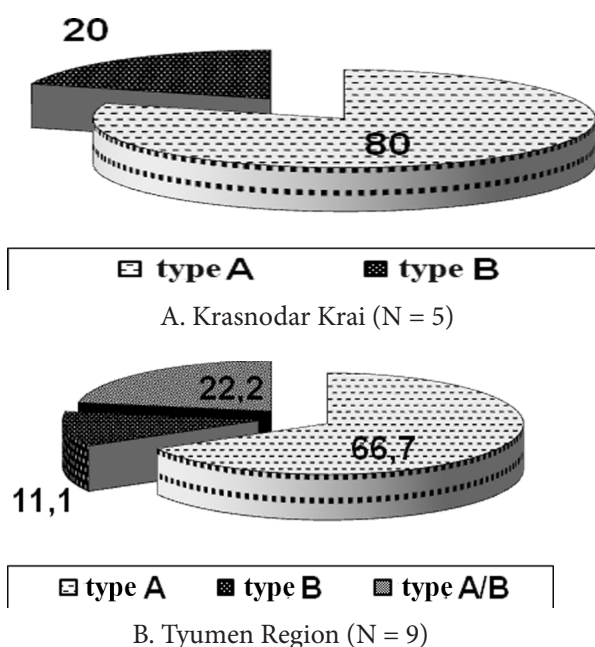


Figure 5. Distribution of BLV Genotypes over the env gene in the Test DNA samples from different regions of Russia (%).

Thus, in the test samples, we predominantly identified samples from the Belgian BLV subgroup (type B) and the Australian subgroup (type A). Approximately 10% of DNA samples can be classified both as the subgroup A and the subgroup B (type A/B) according to distribution of restriction fragments in all endonucleases.

The territorial distribution of various BLV strains has its peculiarities. Presence of mixed genotypes within the same farm or district (Yalutorovsk district of the Tyumen Region) can point to its simultaneous coexistence and

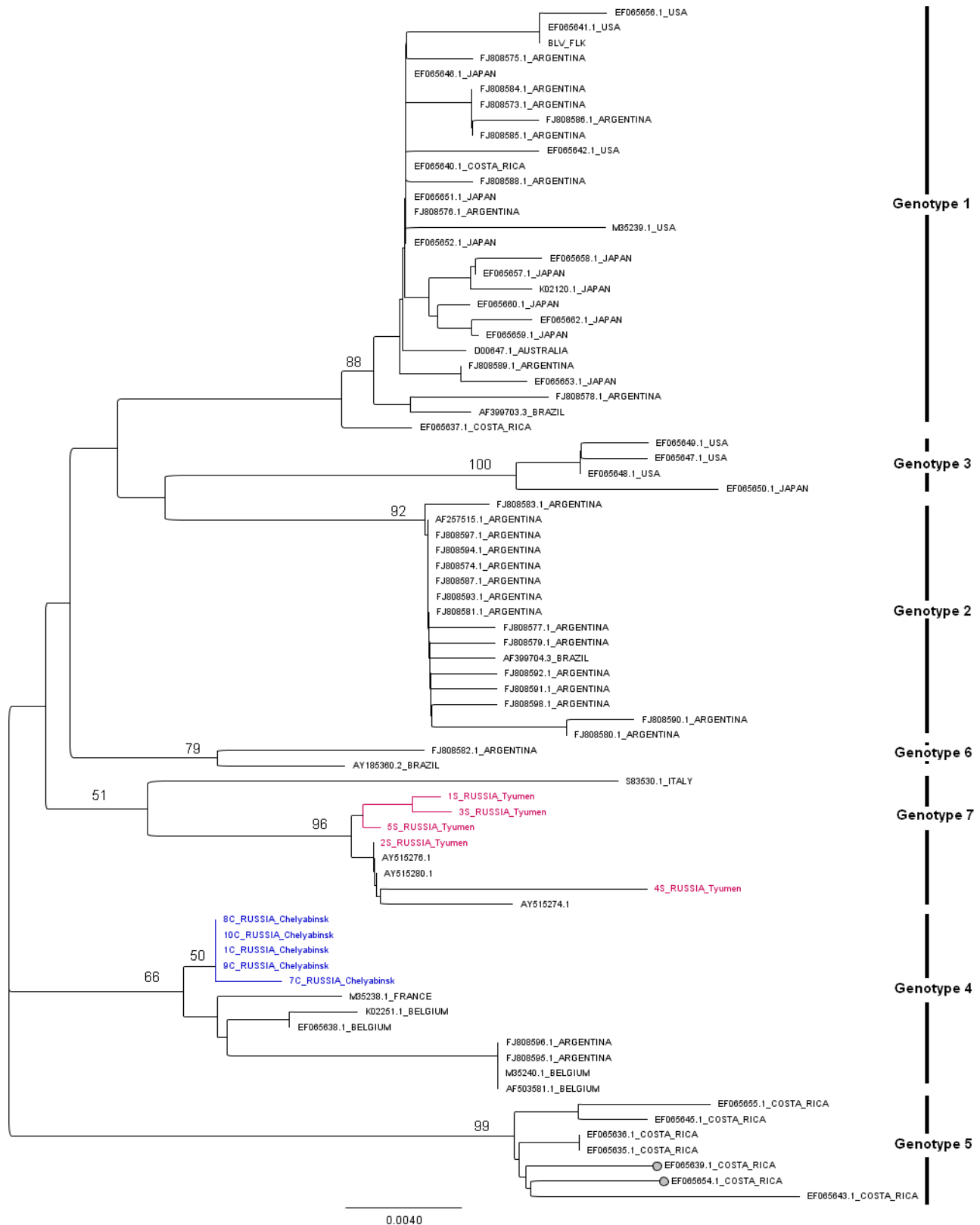


Figure 6. Phylogenetic tree of BLV strains, identified in various countries of the world, including the Ural region of Russia.

possible delivery of one of the BLV types with the imported livestock, due to the lack of officially recommended serological diagnostic methods in the implementation of international and domestic cattle trading. In this regard, we performed DNA-sequencing of the test samples from animals of Chelyabinsk and Tyumen regions.

As a result of the research, we obtained the nucleotide sequences of the DNA of the BLV env 444bp gene, conducted its phylogenetic analysis, which allowed us to classify test strains within the groups and subgroups, to determine its geographic distribution, as well as to compare them with each other and with the world-famous BLV isolates, taken from the International Bank of Genotypes²¹.

In the dendrogram shown in Figure 6, the isolates from the Chelyabinsk and Tyumen Regions are located in the 7 and 4 genotypes, respectively; they formed uniform, but separated branches. When comparing these isolates with the known isolates (FLK_BLV), the most significant changes were made to G, GG, H & D, and ND1, ND2 epitopes.

A large amount of provirus circulates in the blood of such animals, the leukocytosis is observed, the immunological response is suppressed. This “aggressive” type of virus was predominantly defined in the Belgian group, and its existence is explained by the evolutionary stage of the virus adaptation in the host body during the territorial displacement.

Hence, these studies allowed obtaining the additional data on the regional phylogeny of env gene element of Bovine Leukemia. In ²⁴ knowledge of the peculiarities of the structure and functioning of the genome of the virus/provirus will enlarge the fundamental data about the genetics of the virus disease, will hereafter enable not only to understand, but also to control the course of the infection^{25–27} and, probably, will open the ways for the development of vaccines against different types of BLV, thus, improving the health of Russian livestock breeding.

The obtained data are consistent with the works of ²⁸.

4. Conclusions

Extensive studies on the molecular genetic structure of the cattle leukemia virus circulating in the Russian Federation were carried out, and its genotype diversity was determined.

Comparative-bioinformatic analysis of the genome of different BLV genotypes was performed.

The obtained data can serve as a basis for creating highly-sensitive specific primers for efficient PCR diagnostics.

5. References

1. Donnik IM, Petropavlovskiy VM, Tatarchuk AT et al. Sensitivity and specificity of diagnostic tests (ELISA, PCR) in the detection of virus carriers among cattle VL heals from leucosis in cattle. In: Modern problems of diagnostics, treatment and prevention of diseases of animals and birds: Collection of scientific works of leading scientists of Russia and Abroad. Vol. 3. Yekaterinburg: Ural Publishing House; 2010. p. 113–18.
2. Gulyukin MI, Velikhov AF, Nakhmanson VM, Ivanov AL, Grek KP, Lapunov SV. Peculiarities of infectious process induced by leukemia virus in cattle. Proceedings of International Scientific-Practical Conference dedicated to the 110th Anniversary of All-Russian Scientific Research Institute of Experimental Veterinary. Moscow; 2008. p. 106–13.
3. Gladyr EA, Zinovieva NA, Ermilov AA, Vinogradov VN, Ernst LK. Development and pilot testing of a highly sensitive test-system for diagnostics of leukemia virus of cattle. Veterinary Pathology. 2006; 3:87–9.
4. Beier D, Blankenstein P, Marquardt O, Kuzmak J. Identification of different BLV provirus isolates by PCR, and DNA sequencing RFLPA. Berliner und Münchener tierärztliche Wochenschrift. 2001 Jul–Aug; 114(7–8):252–6.
5. Rodriguez SM, Golemba MD, Campos RH, Trono K, Jones LR. Bovine leukemia virus can be classified into seven genotypes: evidence for the existence of two novel clades. Journal of General Virology. 2009, Nov; 90(Pt 11):2788–97.
6. Gulyukin MI, Lomakin NF, Kozyrev NG, Ivanov LA, Klimenko AI, Kovalenko AV. The variability of gene env proviral leukemia cattle circulating in the Rostov Region. Proceedings of All-Russian Scientific-Practical Conference of GNU SKZNIVI RAAS – Novocherkassk; 2009. p. 22–6.
7. Rice NR, Stephens RM, Couez D, Deschamps J, Keltmann R, Burny A, Gilden RV. The nucleotide sequence of the env gene and post-env region of bovine leukemia virus. Virology. 1984; 138(1):82–93.
8. Sagata N, Yasunaga T, Tsuziiku-Kawnmurn J, Ohishi K, Ogawa Y, Ikawa Y. Complete nucleotide sequence of the genome of bovine leukemia virus: its evolutionary relationship to other retro-viruses. Proceedings of the National Academy of Sciences. 1985; 82(3):677–81.
9. Drobot EV. The results of the study of genotypic diversity of the bovine leukemia virus, and epidemiological features and haematological manifestations of leukemia. Author's abstract of PhD Thesis in Biology. Novosibirsk; 2007.
10. Gracheva NI, Smirnov PN. The incidence of leukemia and

- the milk yield of ilirski breed cows of different genealogical lines. Proceedings of International Scientific-Practical Conference Adaptation, Health and Productivity of Animals, Ekaterinburg, 2008 May 22–23; 2008. p.76–8.
11. Mamoun RZ, Morisson M, Rebeyrotter N, Busetta B, Couez D, Kettmann R, Hospital M, Guilemain B. Sequence variability of bovine leukemia virus env gene and its relevance to the structure and antigenicity of glycoproteins. *Journal of Virology*. 1990; 64(9):4180–8.
 12. Fechner H, Kurg A, Blankenstein P, Mewes G, Geue L, Albrecht C, Ebner D. Direct use of cell lysates in PCR-based diagnosis of bovine leukemia virus infection. *Berliner und Münchener tierärztliche Wochenschrift*. 1996; 109(11–12):446–50.
 13. Licursi M, Inoshima Y, Wu D, Yokoyama T, Gonzalez ET, Sentsui H. Genetic heterogeneity among bovine leukemia virus genotypes and its relation to humoral responses in hosts. *Virus Research*. 2002; 86(1–2):101–10.
 14. Asfaw Y, Tsuduku S, Konishi M, Murakami K, Tsuboi T, Wu D, Sentsui H. Distribution and superinfection of bovine leukemia virus genotypes in Japan. *Archives of Virology*. 2005; 150(3):493–505.
 15. Moratorio G, Obal G, Dubra A, Correa A, Bianchi S, Buschiazzi A, Cristina J, Pritsch O. Phylogenetic analysis of bovine leukemia viruses isolated in South America reveals diversification in seven distinct genotypes. *Archives of Virology*. 2010; 155(4):481–9. DOI: 10.1007/s00705-010-0606-3.
 16. Zinovieva NA, Gladyr EA, Bykova AS. Modeling screening systems BOS TAURUS on genetic resistance to bovine leukemia virus. In: current achievements and challenges of agricultural animal biotechnology: the role of nanotechnology in the implementation of the priority tasks of biotechnology 7th international scientific conference-school; 2008. p. 33–40.
 17. Portetelle D, Dandoy C, Burny A, Zavada J, Siakkou H, Gras-Masse H, Drobecq H, Tartar A. Synthetic peptides approach to identification of epitopes on bovine leukemia virus envelope glycoprotein gp51. *Virology*. 1989; 169(1):34–41. DOI: 10.1016/0042-6822(89)90038-X.
 18. Beier D, Blankenstein P, Marquardt O, Kuzmak J. Identification of different BLV provirus isolates by PCR, and DNA sequencing RFLPA. *Berliner und Münchener tierärztliche Wochenschrift*. 2001; 114(7–8):252–6.
 19. Coulston J, Naif H, Brandon R, Kumar S, Khan S, Daniek RC, Lavin MF. Molecular cloning and sequencing of an Australian isolate of proviral bovine leukaemia virus DNA: comparison with other isolates. *Journal of General Virology*. 1990; 71(8):1737–46. DOI: 10.1099/0022-1317-71-8-1737.
 20. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution*. 2007; 24(8):1596–9. DOI: 10.1093/molbev/msm092.
 21. NCBI. National Center for Biotechnology Information [Internet]. Available from: <http://www.ncbi.nlm.nih.gov/guide/genes-expression/>.
 22. Petropavlovskiy MV. Effectiveness of diagnostic tests in the detection of the cattle leukemia virus in herds from leukemia herds. Author's abstract of PhD Thesis in Veterinary. Ekaterinburg; 2010.
 23. Matsumura K, Inoue E, Osawa Y, Okazaki K. Molecular epidemiology of bovine leukemia virus associated with enzootic bovine leukosis in Japan. *Virus Research*. 2011; 155(1):343–48. DOI: 10.1016/j.virusres.2010.11.005.
 24. Bykova AS, Zinovieva NA, Gladyr EA. Rapid diagnosis of BOLA-DRB3alleles associated with cattle resistance to leukemia. *Problems of Biology of Productive Animals*. 2011; 1:12–15.
 25. Vinogradova IV, Gladyr EA, Zinovieva NA. Comparative evaluation of the effectiveness of REID and DNA-diagnostics of leukemia virus of cattle. *Problems of Biology of Productive Animals*. 2011; 1:15–16.
 26. Kuzmak J, Rola M, Kozaczynska B, Pluta A. Bovine Immunodeficiency Virus as a potentiating cofactor for the experimental Bovine Leukemia Virus infection in sheep. *Retrovirology*. 2011 Jun; 8(Suppl 1):1. DOI: 10.1186/1742-4690-8-S1-A5.
 27. Donnik IM. System of measures of rehabilitation of cattle from leukemia in the Urals Federal district. Donnik IM, Smirnov PN, Sivkov GS, Shevkoplyas VN, Shkuratova IA, Tatarchuk AT, editors. Scientific advice, Ekaterinburg: Ural publishing house; 2008. p. 66.
 28. Donnik IM. Regional molecular genetic structure of the cattle leukemia virus / IM Clover, Tatarchuk AT, Lysov AV, Mikheev PM. *Veterinary Kuban. – Krasnodar*. 2010; 3:5–6.