ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Features of the Regional Structure of the BLV Provirus.

Natalya V. Bateneva[1]*, Mikhail I. Voevoda[2], Pavel N. Smirnov[1], Sultan H. Vyshegurov[1], and Mikhail S. Fomenko[1].

[1]Novosibirsk State Agrarian University, 160 Dobrolyubov str., Novosibirsk 630039, Russian Federation.
[2]Research Institute of Therapy and Preventive Medicine, 175/1 Boris Bogatkov str., Novosibirsk 630089, Russian Federation.

ABSTRACT

The aim of our study was to find relationship between changes in the nucleotide sequence of the bovine leukemia provirus structure in cattle of different breed affiliation. The studies were carried out on animals of three breeds: Red Steppe, Holstein Black-and-White, and Ayrshire breed. As a result of conducted research and analysis of restriction maps, 11 pattern groups were identified. Subtypes I-III for each breed were common that confirms the hypothesis of the evolution of viruses depending on breed affiliation. Since all breeds were bred with local cattle, the presence of identical nucleotide sequence is quite understandable.
Keywords: retroviruses, evolution of viruses, bovine leukemia virus, virus typing, regional structure of the virus, adaptive ability of the virus.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The genetic material of viruses is presented in nature in the following forms: single- stranded and double-stranded RNA and DNA, as well as their linear, circular and fragmentary species. Evolutionary formed options of the genetic material of viruses' predecessors are rather diverse; at present, generally accepted are canonical forms, i.e. double-stranded DNA as the genetic information bearer and a single-stranded RNA as its transmitter. The diversity of genetic material in viruses is more like evidence of polyphyletic origin of viruses, rather than the preservation of ancestral precellular forms, whose genome evolved via the unlikely path from RNA to DNA, from single-stranded forms to double-stranded ones and so on [3, 5, 7, 9, 10, 14, 31, 36].

The development of molecular genetics has materialized the concept of the gene, as well as chemical basis of mutations and recombination (including point mutations, indels, deletions, and rearrangements). However, molecular genetics well explained just the microevolution processes, mainly to the extent of world, though not placed in the clearest light the macroevolution processes - formation of large taxonomic groups, which are the basis of progressive evolution [3, 5, 7, 9, 20, 31, 36].

To explain the molecular basis of these processes, as well as the actual pace of evolution, a theory of genes and genomes duplication was proposed. This concept goes with observed facts and explains quite well the evolution of the organic world on Earth, in particular, the emergence of the vertebrate (chordate) and their further evolution from primitive cranial animals to human beings. As a result, the concept has rapidly gained acceptance among biologists, who study molecular basis of evolution.

A significant number of accumulated facts testify the possibility of extensive exchanging in nature of ready-made blocks of genetic information, which includes also the representatives of different evolutionary distant viruses. As a result of this exchange, hereditary properties can quickly and abruptly change by incorporation of foreign genes (borrowing gene function). New genetic quality can also occur due to unexpected combination of inherent and integrated genes (the emergence of a new function). Finally, a simple increase in genome owing to non-functional genes opens the possibility for their evolution (the formation of new genes).

A special role in facilitating these processes belongs to viruses, which are autonomous genetic structures that include both conventional viruses and plasmids. This hypothesis was first proposed in general terms, and then thoroughly elaborated [5, 9, 11, 31, 36]. The main idea of the hypothesis was the recognition of viruses as intracellular (genetic) "parasites", though qualifying them as important factors of organic evolution not only in the early stages of evolution (temperate phages, plasmids), but in the later (retroviruses) stages of evolution. Involvement of viruses allows explaining some facts of detection of the same genes in evolutionary distant taxonomic groups. Figuratively speaking, viruses are a tool for sharing best practices in the biosphere [3, 7, 9, 36].

Among retroviruses known to humankind, we have identified the one, which is different from all known C-type viruses of mammals, though has a resemblance to human T-lymphotropic virus type I, whose natural carrier is cattle. Bovine leukemia virus (BLV) – is the exogenous oncovirus type C, which is integrated into the genomic DNA of B-lymphocytes as an intermediate DNA form (the provirus) [27-29, 31, 32].

The surface of the virus outer shell has peplomers, which consist of subunits formed by two glycosylated viral proteins *gp51* and *gp30* responsible for type specificity [12, 17, 31, 35].

Six major proteins (*p10, p12, p15, p24, gp30* and *gp51*) were found out in the structure of BLV [18, 25-27, 31, 32, 35].

The core virion consists of *p10, p12, p15,* and *p24* proteins, which belong to non-glycosylated proteins. At that, the highest protein content is accounted for *p24* [25, 28, 31].

The *Gp51* is located on the virion surface, *gp30* – is transmembrane protein; both the first and the second proteins are complex ones belonging to glycoproteins [25, 31].

ISSN: 0975-8585

The BLV has a pronounced antigenic activity. The *gp51* protein, in which 3 epitopes were revealed, can neutralize the virus and is responsible for infectivity and antigenic activity.

Poly- and mono-antibodies against *gp51* have intracellular activity and inhibit syncytium forming activity of the virus and its efflux out of cells as well as cause lysis of infected cells in the presence of complement [25].

The BLV envelope contains 2 *gp60* proteins, surface glycoprotein and *p30*-intermembrane protein. The genome of the virus can exist in two forms:

- Genomic single-stranded RNA;
- In the form of DNA synthesized on the genomic RNA as the matrix and integrated into the chromosome of the «host» cell in the form of a provirus (Fig. 1) [25].

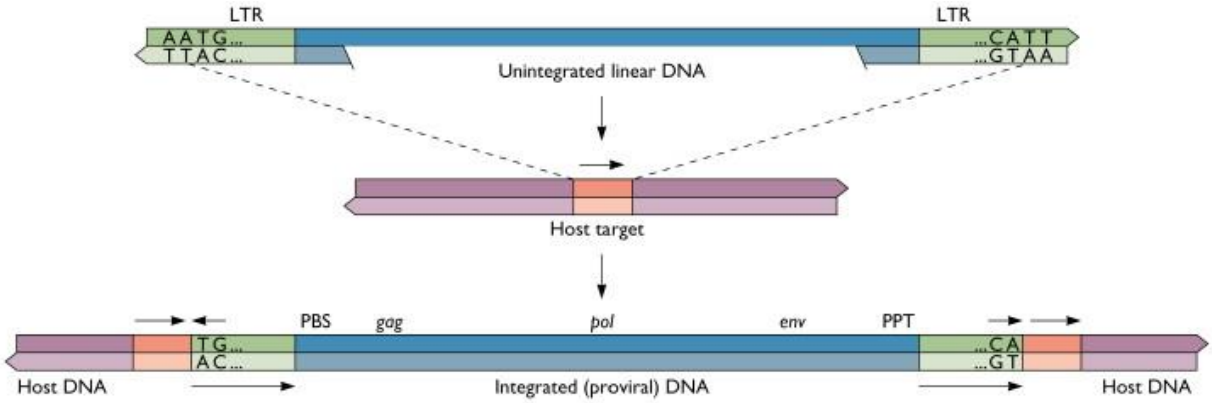

Figure 1. Scheme of the virus integration into the cell.

The genomic RNA of the virus is contained only in the mature virions [21, 22, 25, 31]. In the life cycle of the virus, genomic RNA functions just once when it serves a matrix for enzyme – the reverse transcriptase in the biosynthesis [24, 25, 31].

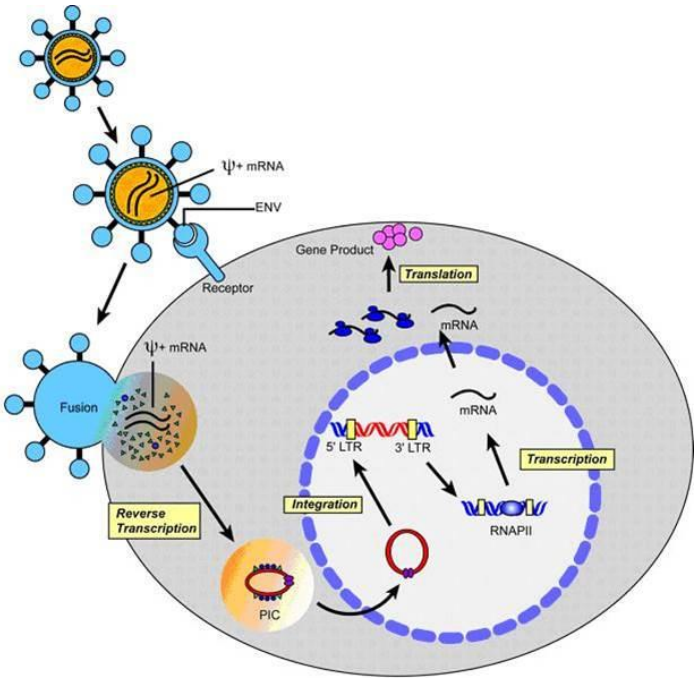

Figure 2. Scheme of the replication-competent retrovirus life cycle [9, 25].

The entire volume of the biochemical processes required for full virus replication is performed with the involvement of DNA-provirus.

Genome consists of *8714* nucleotide sequences and has the following genetic maker: *5'LTR-gag-pol-env-pX(BL)-3'LTR*. The interaction mechanisms of the bovine leukemia virus in the "virus-cell" system, which currently remain unclarified, are of particular interest [1, 8, 10, 11, 12, 25, 33]. Thus, after experimental infection of calves with the BLV, the virus is eliminated from the animal body in 50% of cases, while in some animals of the remaining 50% it causes leukemia, which transforms over time from hematological stage (PL-lymphocytosis) into clinical stage [19, 23, 25, 30].

It is known that the cattle meat breeds are non-susceptible to the BLV; however, the mechanism of animals' protection against this virus still remains unclear [2-4, 31]. Perhaps, it is in absconded (inactive) state. Besides, viruses naturally undergo evolutionary change, adapting to the «host» body. As a result of these changes, at this time the BLV is classified in 7 types (depending on origin) [1, 6, 9, 10, 13, 15, 16, 20]. Over the past 5 years there have been reports about the existence of 8th BLV genotype [34].

After analyzing the overall scheme of the life cycle of retroviruses, and the BLV in particular, we came to the following conclusion: there are two control phases in the interaction of virus cell with the «host» cells. The first phase concerns the interaction of the viral envelope with the animal's cellular membrane. At that, this stage involves the *env* gene, whose structure has been widely studied throughout the world. The second phase consists in the interaction of the animal's cell nucleus with the virion. This process involves proteins encoded by the *gag* gene (Fig. 2).

Because the nucleotide and amino-acid substitutions, identified in the genome of different BLV types and subtypes on the patch, encoded by the *env* gene, cannot be differentiated by sensitivity to certain cattle breed affiliation, we chose the *gag* gene as the gene under investigation.

The restriction analysis data, previously obtained for a small area (347 bps) of *gag* gene, has shown that there is a certain correlation between breed affiliation of the animal and pattern changing of the BLV circulating in the animals of specifically studied breed [9].

METHODS

The research object were the cows of different breed affiliation from joint stock companies and breeding farms of Krasnodar and Stavropol regions, namely Red Steppe, Holstein Black-and-White, and Ayrshire breeds. Three hundred blood samples were taken based on random sampling technique (100 samples from each farm) from the animals reacting to the BLV in immunodiffusion test, *gp51*.

The research subjects were above mentioned blood samples taken from caudal vein of the tested animals and sampled into sterile glass tubes.

Further, DNA was isolated from blood samples by the sorbent method with the use of "AmpliSens" kits for isolation of nucleic acid in accordance with the attached manual.

For the selection of the oligonucleotide primers we have analyzed 11 isolates of the BLV from the International NCBI GI database: 151413539, 210767, 237688388, 9739119, 678246211, 678246204, 429534208, 429534201, 429534194, 9626225, and 2801494.

Considering substitutions in the patterns of some well-known isolates, we have designed primers flanking the variable region of the *gag* gene (Fig. 3).

$^{230\text{-}323}$5′-GGGCGTCTGGCTTGCACCCG-3′
$^{1570\text{-}1575}$5′- CCCATTGGAAACGRGACTGT -3′

ISSN: 0975-8585

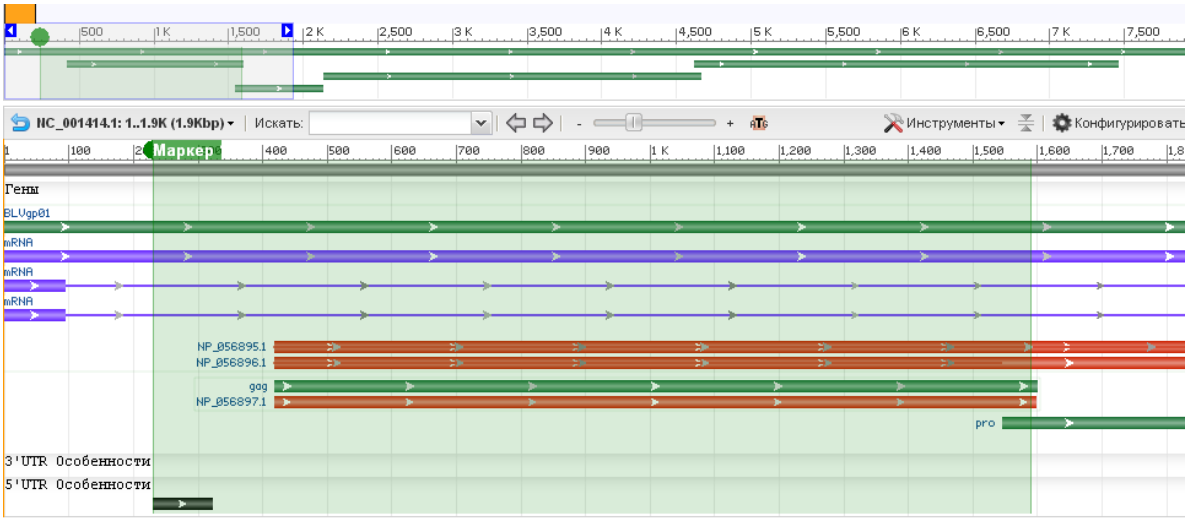

Figure 3. Diagram of the BLV provirus patch, flanked by oligonucleotide primers.

The RialTime PCR reactions triplet was conducted using the SuberGreen dye. After receiving the data, the melting temperature of the primers was determined; it was 68°C. A control reaction was carried out using the calculated temperature profile.

Temperature profile was as follows: 2 min – 94°C; (30 s – 95°C; 30 s – 68°C; 30 s – 72°C) – 40 cycles; 3 min – 72°C.

These primers capture the entire *gag* gene (a small part of the LTR-region) (Fig. 3) with all known substitutions; they are sensitive to the sequences of all currently known BLV (NCBI) types and subtypes.

LTRCCAAGGGCGTCTGGCTTGCACCCGCGTTTGTTTCCTGTCTTACTTTCTGTTTCTCGCGG↑CCCGCGCTCTCTCCTTCGGCGCCC
TCTAGCGG↑CCAGGAGAGACC↑GGCAAACAATTGGGGGCTCGTCC↑GGGATTGATCACCCC↑GGAACCCTAATAACTCTCTGGACC
CACCCCCTCGGCGGCATTTTGGGTCTCTCCTTCAAATTATATY^GAGATG↑GGAAATTCCCCYTCYTATAACCCYCCCGCTGGTATCT
CCCCCTCAGACTGGCTCAACCTTCKKCAAAGCGCGCAAAGGCTCAATCCGCGACCCTCTCCTYAGCGATTTTACCGATTTAAARAA
TTACATCCATTGGTTTYATAAGACCCAGAAAAAACCATG↑GACTTTCACTTCTGGTRG↑CCCCRCCTCATG↑YCCACCC↓GGGARA
TTCGG↑YC↓GGGTTCCCCTYGTCYTGG↑CCACCCTAAACGAAGTRCTCTCAAACGAWGRRGGCRCCCC↓RGGTGCATCRG↑CCCCA
GAAGAACAACCCCCCCCTTATGACCCCCCCGCYRTTYTGCCAATYATATCTGAAGGRAATCGYAACCGCCATCGYGCYTGGGCACT
CCGAGAAKTACAAGATATYAAAAARGAAATTGAAAATAAGGCRCC↑GGGTTCGCAAGTATGGATACAAACACTACGRCTTGCAATC
YTRCAGG↑CCGACCCTACTCC↓KGCTGACCTAGAACAACTTTGCCAATATATTGCTTCCCC↓GGTCGAYCAAACGG↑CCCAYATGA
CYAGCCTAACGGCAGCAATAGYCGCCGCTGAAGCGG↑CCAAYACCCTCCAGGGTTTT(-)AAYCCCCAAAAYGGGNACCCTRACCC
AACAATCAGCTCAGCCCAACGCC↓GGGGATYCTTAGAAGTCAATATCAAAACCTYTGGCTTCARG↑CYTKGAAAAATCTCCCTACT
CGK↑CCTTCAGNTVCAACCYTGGTCCACCATCGTCCAAGG↑CYCCGCCGARAGCTMTGTAGARTTTGTCAACC↓GGTTACAAATTT
CATTAGCTGACAACCTTCCCGACGGAGTCCCTAARGAACCCATTATTGACTCYCTTAGYTATGCWAATGCTAACARAGARTGYCAR
CAAATTTTGCARGGGCGRGG↑CCNAGTGG↑CCGCC(-)C(-)C(-)↓GGTGGGG(A)CAAAAACTGCAGGCTTGYGCACA-(A)TT
GGG↑CCCCCAAGR-(A)TBAAACAGCCTGCRVTYCTCGTCCACACCCCAGGG↑CCAAGATGCCYGGG↑CCYC↓GGCAACCGG↑CC
CYCCAAAAGG↑CCYCCCCCRGGACCATG↑CTATCGATGCCTCAAAGAAGG↑CCATTGGG↑CCC↓GGGAYTGTCCYACCAAGRCCAC
C↑GG↑CCCMCCTCC↓GGGACCTTGYCCYATATGTAAAGATCYTTCCCATTGGAAACGRGACTGTCCAACCCTCAAATCAAAAAACT

Figure 4. Location diagram of known nucleotide substitutions in the BLV provirus on the patch of the genome (which includes the *gag* gene and part of the LTR-region), flanking by GGCGTCTGGCTTGCACCCG and CCCATTGGAAACGRGACTGT primers with indication of restriction sites by endonucleases *Fae I* (CATG↑, ↓GTAC) and *Hae III* (GG↑CC, CC↓GG) .

Given restriction map (Fig. 4) allows stating the structural features of the 11 pattern groups (isolates) of the provirus. However, *some spectrotypes* are peculiar to only particular cattle breed that gives grounds to speak about possible adaptation of the virus to the characteristics of a carrier - bovine cattle [3, 5].

ISSN: 0975-8585

DISCUSSION AND RESULTS

Comparing the visualization data of the results obtained from electrophoregrams with the expected products of hydrolysis and combining them, we conducted a conditional division of the BLV into 11 pattern groups, which distributed as follows (Fig. 5).

- Three BLV subtypes (I, II, III) circulate among bovine cattle of Red Steppe breed with a predominance of genotype III.
- Six BLV subtypes (*gag* gene) from I to VI with a predominance of I were identified in Holsteins animals.
- Eight BLV subtypes – I, II, III, VII, VIII, XI, X, and XI, with a predominance of I were identified in the Ayrshire cattle breed from the "V.I. Chapaev Breeding Farm" (Table 1).

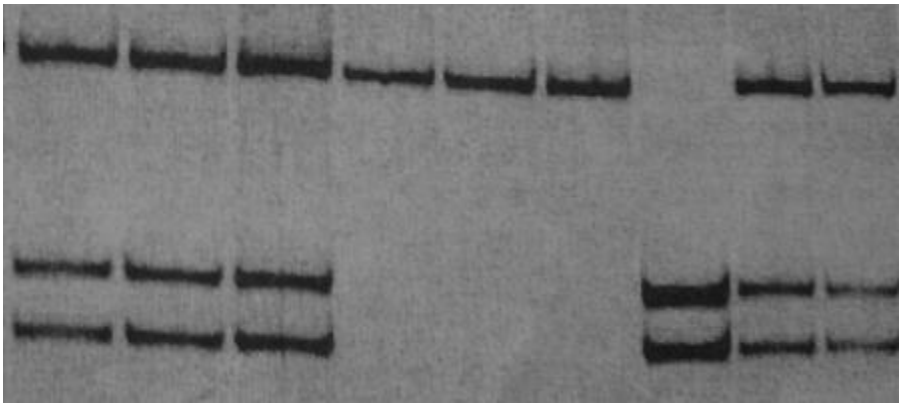

Figure 5. Electrophoretogram of products of hydrolysis (polyacrylamide gel) conducted by endonuclease of *Fae I* restriction of the amelification patch of the *gag* gene (347 bps). Investigated DNA corresponds to the BLV provirus circulating in the Red Steppe cattle breed. Lanes numbering from left to right from 1 to 9; 1-3, 8, 9 – I group of patterns; 4-6 – II group of patterns; 7 –III group of patterns.

Table 1: The distribution of the BLV subtypes in the farms of Krasnodar Territory

Farm, breed	The BLV (*gag*) subtype (group of patterns), %										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
CJSC "Agronom", Red Steppe breed	17	8	75	-	-	-	-	-	-	-	-
Agricultural production cooperative "Kolos", Black-and-White Holstein breed	44	12	6	6	13	19	-	-	-	-	-
V.I. Chapaev Breeding Farm, Ayrshire breed	40	6	6	-	-	-	7	20	7	7	7
Total	101	26	114	6	13	19	7	20	7	7	7

According to the data presented in the professional literature, non-uniform distribution of the BLV genotypes in the Krasnodar Territory maybe has resulted from evolutionary changes in the nucleotide sequence of the virus in the course of adaptation to the "host" genome.

However, it should be noted that three provirus genotypes are common to the BLV-infected cows from all investigated farms. Therefore, we can assume that the other BLV genotypes were introduced with imported livestock, since screening of imported animals for leukemia was not carried out before the 80-ies of the previous century. Later on screening was conducted only through the immunodiffusion test, which has quite low sensitivity, especially in the early stages of infection.

CONCLUSION

The bovine leukemia virus, which circulates on the Krasnodar Territory of the Russian Federation, is genetically heterogeneous. Although the virus structure did not undergo significant changes and individual

virus genotypes were not detected, there were changes in the provirus genome, caused, apparently, by the peculiarities of the "host" breed affiliation.

Changes that were revealed in the structure of the provirus genome can be grouped based on the RFLP profiles. Eleven pattern groups, which in varying degrees are distributed in the breeding farms of the Krasnodar Territory, were visualized on electrophoregrams.

Further research in this area will allow most comprehensive understanding the nature of the BLV and optimizing the program of curative measure in herds affected by this infection.

REFERENCES

[1] Alvarez, I., Gutierrez, G., Gammella, M., Martinez, C., Politzki, R., Gonzalez, C., Caviglia, L., Carignano, H., Fondevila, N., Poli, M., and Trono, K., 2013, "Evaluation of Total White Blood Cell Count as a Marker for Proviral Load of Bovine Leukemia Virus in Dairy Cattle from Herds with a High Seroprevalence of Antibodies against Bovine Leukemia Virus," Am. J. Vet. Res., 74, pp. 744–749.

[2] Blood, D.C., Henderson, J.A., and Radostits, O.M., 1979, "Leucosis in Cattle and other Species," Veterinary Medicine, 5th ed., London, p. 611. ISBN 0-7020-0718-8.

[3] "Bovine Leucosis Virus on U.S. Dairy Operations," 2007, NAHMS Dairy, U.S. Department of Agriculture.

[4] Bovine Leukemia Virus reviewed and published by WikiVet, 2011.

[5] Buehring, G.C., Philpott, S.M, Choi, K.Y., 2003, "Humans Have Antibodies Reactive with Bovine Leukemia Virus," AIDS Res. Hum. Retroviruses 19(12), pp. 1105–1113. DOI:10.1089/088922203771881202. PMID 14709247.

[6] Burny, A., Bruck, C., Cleuter, Y., Couez, D., Deschamps, J., Gregoire, D., Ghysdael, J., Kettmann, R., Mammerickx, M., and Marbaix, G., 1985, "Bovine Leukaemia Virus and Enzootic Bovine Leukosis," J. Vet. Res., 52, pp. 133–144.

[7] Smirnov, P.N., Garmatarova, T.V., Bateneva, T.V. et al., 2013, "Comparative Biochemical Blood Parameters of Ayrshire Breed Cows due to their Productivity and Trouble on BLV Infection," Intern. B. I. Phys., 9. pp. 64-66.

[8] Donnik, I.M., Dailide, G.A., Yakubenko V. E., and Tikhonov, S.V., 2014, "Epizootic Aspects of Bovine Leukemia in the Krasnodar Region," Veterinary Kuban, 2, pp.15-18.

[9] Gracheva, N.V., 2010, "Genotypic Diversity on BLV *gag* and *env* Genes in Cattle of Different Breeds," Ph.D. thesis, Institute of Experimental Veterinary Science of Siberia and the Far East, Novosibirsk.

[10] Gutierrez, G., Carignano, H., Alvarez, I., Martinez, C., Porta, N., Politzki, R., Gammella, M., Lomonaco, M., Fondevila, N., Poli, M., and Trono, K., 2012, "Bovine Leukemia Virus *p24* Antibodies Reflect Blood Proviral Load," BMC Vet. Res., 8, p. 187.

[11] Jimba, M., Takeshima, S.N., Murakami, H., Kohara, J., Kobayashi, N., Matsuhashi, T., Ohmori, T., Nunoya, T., and Aida, Y., 2012, "BLV-CoCoMo-qPCR: a Useful Tool for Evaluating Bovine Leukemia Virus Infection Status", BMC Vet. Res., 8, p.167.

[12] Johnson, E.S., 2005, "Assessing the Role of Transmissible Agents in Human Disease by Studying Meat Workers", Cell Science Reviews, 2(1), ISSN 1742-8130.

[13] Kabeya, H., Ohashi, K., and Onuma, M., 2001, "Host Immune Responses in the Course of Bovine Leukemia Virus Infection," J. Vet. Med. Sci. 63 (7), pp. 703–708, DOI:10.1292/jvms.63.703.PMID 11503896.

[14] Kettmann, R., Burny, A., Callebaut, I., Droogmans, L., Mammerickx, M., et al., 1994, "Bovine Leukemia Virus," In: The Retroviridae, New York, Plenum Press. pp. 39–81.

[15] Licursi, M., Inoshima, Y., Wu, D., Yokoyama, T., and Gonzalez, E.T., 2002, "Genetic Heterogeneity among Bovine Leukemia Virus Genotypes and its Relation to Humoral Responses in Hosts," Virus Res. 86, pp. 101–110, DOI: 10.1016/s0168-1702(02)00059-x

[16] Mammerickx, M., Portetelle, D., de , K., and Burny, A., 1987, "Experimental Transmission of Enzootic Bovine Leukosis to Cattle, Sheep and Goats: Infectious Doses of Blood and Incubation Period of the Disease," Leuk. Res., 11, pp. 353–358.

[17] Mechanisms of Leukemogenesis Induced by Bovine Leukemia Virus: Prospects for Novel Anti-retroviral Therapies in Human," Retrovirology, 4(18), DOI:10.1186/1742-4690-4-18. PMC 1839114. PMID 17362524.

[18] Mewes, G., 1997, "Langzcitstudie zur Persistent des Bovinen Leukose-virtrs (BLV) in einer leukosesanierten Milchviehhcrde," Vet. Diss., Berlin.

[19] Sagata, N., Yasunaga, T., Tsuziiku-Kawnmurn, J., Ohishi, K., Ogawa, Y., and Ikawa, Y., 1985, "Complete Nucleotide Sequence of the Genome of Bovine Leukemia Virus: Its Evolutionary Relationship to other Retro-Viruses," Proc. Natl. Acnd. Sci. USA 82, pp. 677-681.

[20] Smirnov, A.M., Gulykin, M.I., Subbotin, V.V., Donnik, I.M., Kolbasov, D.V., Skhirat, V.N., Suvorov, A.V., Babyshow, L.V., and Sribnyi, N.A., 2008, "New Methods of Research on Problems of Veterinary Medicine," Part IV, Laboratory Methods for Studies of Infectious Diseases of Animals, Moscow.

[21] Rosewick, N., Momont, M., Durkin, K., Takeda, H., Caiment, F., Cleuter, Y., et al., 2013, "Deep Sequencing Reveals Abundant no Canonical Retroviral MicroRNAs in B-cell Leukemia/lymphoma". Proceedings of the National Academy of Sciences, 110(6), pp. 2306–2311, DOI: 10.1073 /pnas. 1213842110. PMC 3568357. PMID 23345446.

[22] "Enzootic Bovine Leucosis (PDF)," 2010, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. World Organisation for Animal Health (OIE), Chapter 2.4.11.

[23] Petropavlovskiy, M., Donnik, I.M., Tatarchuk, A.T., Lysov, A.V., Vovchenko, Y.F., Mikheev, P.M., 2010, "Peculiarities of the Course and Distribution of Bovine Leukemia in the Southern Urals," Phys. Agrarian Bulletin of the Urals, Ekaterinburg, 10, p. 48.

[24] Donnik, I.M., and Dailide G.A., 2013, "Prevention of Bovine Leucosis in Breeding Farms of the Krasnodar Territory," Veterinary Kuban, 5, pp. 8-10.

[25] Donnik, I.M., and Petropavlovskiy, M., 2010, "Regional Molecular Genetic Structure of the Virus Leukemia in Cattle," Veterinary Kuban, 3. pp. 12-13.

[26] Coffin, J.M., Hughes, S.H., and Varmus, H.E., 1997, "Retroviruses," Cold Spring Harbor Laboratory Press.

[27] Rice, N.R., Stephens, R.M., Burny, A., and Gilden R.V., 1985, "The *gag* and *pol* Genes of Bovine Leukemia Virus," Nucleotide Sequence and Analysis, Virology, 142, pp. 357–377. [PubMed]

[28] Rice, N.R., Stephens, R.M., Couez, D., Deschamps, J., Kettmann, R., Burny, A., and Gilden, R.V., 1984, "The Nucleotide Sequence of the *env* Gene and post-*env* Region of Bovine Leukemia Virus," Virology ,138, pp. 82–93. [PubMed]

[29] Sagata, N., Yasunaga, T., Tsuzuku-Kawamura, J., Ohishi, K., Ogawa, Y., and Ikawa, Y., 1985, "Complete Nucleotide Sequence of the Genome of Bovine Leukemia Virus: Its Evolutionary Relationship to other Retroviruses," Proc. Natl. Acad. Sci., 82, pp. 677–681. [PubMed]

[30] Seiki, M., Hattori, S., Hirayama, Y., and Yoshida, M., 1983 "Human Adult T-cell Leukemia Virus: Complete Nucleotide Sequence of the Provirus Genome Integrated in Leukemia Cell DNA," Proc. Natl. Acad. Sci., 80, pp. 3618–3622. [PubMed].

[31] Smirnov, P.N., 2007, "The Disease of the Century - the Bovine Leukemia," Novosibirsk.

[32] Syurin, V.N., Samuylenko, A.Ya., Solovyov, B.V., and Phomina, N.V., 1992, "Viral Diseases of Animals," Moscow, 928 p.

[33] Takatori, I., Itohara, S., and Yonaiyama, K., 1982, "Difficulty in Detecting in vivo Extracellular Infective Virus in Cattle Naturally Infected with Bovine Leukemia Virus," Leuk. Res. 6(4), pp. 511–517, DOI:10.1016/0145-2126(82)90008-X. PMID 6292585.

[34] Donnik, I.M., Shkuratova, I.A., Shusharin, A.D., Vereshchak, N. A.., and Belkin, Y.B., 2007, "The Influence of Environmental Factors on the Organism of Animals," Veterinary Medicine, 6, pp. 38-42.

[35] Rola-Łuszczak, .Y., Pluta, M., Olech, A., Kuźmak, M., Donnik, J., Petropavlovskiy, I., Gerilovych, M., Vinogradova, A., Choudhury, I., 2013, "The Molecular Characterization of Bovine Leukaemia Virus Isolates," PLoS ONE, 8(3), 58705.

[36] Willems, L., Thienpont, E., Kerkhofs, P., Burny, A., Mammerickx, M., et al., 1993, "Bovine Leukemia Virus an Animal Model for the Study of Intrastrain Variability," J. Virol. 67, pp. 1086–1089.

[37] Zhdanov, V. M., 1990, "Evolution of Viruses," Moscow, Medicine, 376 p.