WebLog Journal of Veterinary Science and Animal Husbandry

Research Article

Published: 11 May, 2026

Using RT-PCR Method for Accelerated Health Improvement of Herd of Cows from Leukemia

Irina Ya. Nam[1]*, Vladimir V. Zayakin[2], Roman B. Achmedov[2], Tachir M. Achmetov[3] and Olga I. Solovjeva[4]

[1]Association "Agrobiotechnology and Digital Systems in Agriculture", Leningrad Region, Volkhovsky District, Russian

[2]Bryansk State University Named After Academician I.G. Petrovsky, Bryansk, Russia

[3]Kazan State Agrarian University, Republic of Tatarstan, Kazan, Russia

[4]Russian State Agrarian University, Moscow Timiryazev Agricultural Academy, Timiryazevskaya St., Moscow, Russia

OPEN ACCESS

***Correspondence:**
Dr. Irina Ya. Nam, Association "Agrobiotechnology and Digital Systems in Agriculture", Leningrad Region, Volkhovsky District, Russian, Tel: +79214248503;
E-mail: iyanam1@yandex.ru/ORCID: https://orcid.org/0000-0003-3572-2815

Received Date: 15 Apr 2026
Accepted Date: 09 May 2026
Published Date: 11 May 2026

Citation:
Irina Ya. Nam, Zayakin VV, Achmedov RB, Achmetov TM, Solovjeva OI. Using RT-PCR Method for Accelerated Health Improvement of Herd of Cows from Leukemia. WebLog J Vet Sci Anim Husb. wjvsah.2026.e1101. https://doi.org/10.5281/zenodo.20177437

Copyright© 2026 Dr. Irina Ya. Nam. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

The spread of enzootic leukemia is a global problem of cattle breeding

The leukemia virus causes blood disease in 29 species of animals and 15 species of domestic and wild birds. Bovine enzootic leukemia (international designation of bovine leukemia) is included in the list of diseases of the World Organization for Animal Health, as a disease subject to mandatory notification (notification to the international community). The high prevalence of bovine leukemia virus in cattle herds in livestock farms represents a danger to the whole world, threatening not only cattle breeding, but also public health. The infection rate of animals with leukemia in large farms in a number of countries reaches 85%, and in some cattle breeds in the USA it is about 50%. In Russia, leukemia firmly occupies the first place among infectious diseases of cows.

When infected, the proviral DNA is embedded in the genome of blood cells. The development of the disease has a long incubation period of 5-6 years, which is asymptomatic, which, in the absence of proper measures to combat leukemia, can lead to the uncontrolled spread of the virus in herds of cows. Clinical signs of leukemia in sick animals appear several years after infection: enlarged lymph nodes, the appearance of tumors, weight loss, decreased productivity. The appearance of symptoms of the disease has no diagnostic value, since they manifest themselves only at the terminal stage, ending within a few weeks with the death of the animal [1]. Enzootic leukemia in cows is not being treated, and the identified sick animals must be immediately culled.

Social problems of widespread enzootic leukemia

The problem of leukemia in cattle is of great social importance, since metabolites that are oncogenic substances accumulate in the tissues of sick animals, and milk from leukemic cows cannot be used for the production of baby food.

Of great potential danger is the fact that retrovirus type C, which causes leukemia in cows, is structurally and functionally similar to the HTLV-I and HTLV-II viruses, which cause T-cell leukemia in humans (HTLV types 1 and 2), however, unlike it, it affects B lymphocytes. The genome structures of human T-cell leukemia provirus and BLV are similar, and a certain homology of the genes of both viruses encoding the internal structural protein has been established [8]. Analysis of the genomes of BLV and human T-cell leukemia virus performed by [15], suggests that these viruses originated from a common precursor. There are no oncogenes in the genomes of BLV and HTLV, and no preferred sites for the integration of BLV and HTLV proviruses into the genome of the host cell have been identified.

Due to the high level of genetic homology with the human T-cell leukemia virus, there is a risk of human infection. Retroviruses can be inducers of tumor diseases in humans and animals, such as leukemia, breast tumors, or skin cancer - the virus has been detected in 80% of women with breast cancer, and it has been detected in children with blood cancer [19].

Methods for diagnosing cow leukemia and identification of the pathogen virus

Immunological studies to detect BLV-infected animals: Currently, immunological methods such as RID (radial immunodiffusion) and ELISA (enzyme immunoassay), hematological and

molecular genetic methods are used to diagnose bovine leukemia and identify its causative agent.

Immunological studies to identify animals infected with BLV: When the pathogen enters the animal's body, it is accompanied by the production of specific antibodies to viral proteins; in infected animals, antibodies against gp51 (envelope proteins) and p24 (to gag proteins) circulate in the blood.

In Russia, a double annual examination of cattle by radial immunodiffusion (RID) is carried out. RID is a priority diagnostic method for BLV, used to identify farms with leukemia problems. The RID method has a relatively low specificity and sensitivity.

Enzyme-linked immunoassay (ELISA) is based on the use of enzymatically labeled antibodies, which make it possible to diagnose antibodies or antigens in various biological fluids of cattle [3, 20]. This method has 10-100 times more sensitivity than RID. This method implies a high degree of automation at all stages, which reduces the subjective impact on its outcome.

Serological tests cannot be applied to detect infected young animals up to the age of six months and pregnant cows in the last two months before calving, since the presence of maternal (colostral) antibodies in the blood of calves can lead to false seropositive tests [9]. As a result, in the first 6 months before the RID, calves are reinfected and virus carriers in the repair herd increase several times – from 2-6% to 25%.

The impossibility of early detection of all BLV-infected calves by the RID method leads to a delay in the process of improving the number of cows on farms. For economic reasons, the culling of 25% of repair RID+ heifers is difficult, so there is an increase in infected and sick cows in the herds.

Hematological examination to detect bovine enzootic leukemia

The hematological method determines the presence of the BLV infectious process at the stage of persistent lymphocytosis, when microscopic examination of a blood smear shows an increase in the total number of leukocytes and a change in the leukocyte formula [18]. A typical sign of lymphocytosis is an increased number of lymphoid cells of varying degrees of maturity, due to the active proliferation of infected lymphoid tissue. Weakly differentiated, polymorphic cells appear in the peripheral blood. Mature lymphocytes are observed in the chronic course of the disease, exacerbation leads to the appearance of juvenile forms [7, 14]. Persistent lymphocytosis is diagnosed only with prolonged and sustained persistence of hematological changes.

Usually, hematology analysis is not used for mass screening. It is carried out on blood samples of RID-positive cattle in order to cull heme-diseased animals. There is a correlation between the number of antibodies and hematological manifestations of leukemia.

Molecular genetic methods for the diagnosis of BLV-infected animals.

Currently, much attention is being paid in Russia and the world to the development of various polymerase chain reaction (PCR) methods for the diagnosis of infectious and hereditary diseases. These studies became particularly relevant in 2020 in connection with the COVID-19 pandemic, an acute respiratory infection caused by the SARS-CoV-2 (2019-nCoV) coronavirus [22].

PCR is based on multiple copying of a specific fragment of a target DNA molecule until a detectable concentration is reached [16]. PCR has a high (up to 100%) sensitivity and specificity. Thus, the use of the PCR method makes it possible to increase the number of detected virus carriers by 10-42% in comparison with RID and ELISA. The PCR diagnostic method allows direct detection of foreign DNA, determining the presence of the infectious agent.

Most of the methods of BLV PCR diagnostics are based on the detection of the pathogen's proviral DNA in the animal's genomic DNA. The specificity of BLV detection is determined by the choice of primers complementary to the known nucleotide sequences of the virus genes.

To diagnose BLV, the pol and env gene sequences are used, which are characterized by low polymorphism, which makes it possible to use one universal pair of primers to identify all viral biotypes. The diagnostic advantages of the envelope protein gene include its high preservation without deletions even in tumor cells [12]. Most often, fragments of the pol and env genes are found in the genomic DNA of cattle.

At the stage of detection of PCR amplification products of BLV provirus, electrophoretic separation of amplified fragments in an agarose or polyacrylamide gel with UV imaging in an intercalating dye is used, the specificity of the product is determined by its length [4, 5].

However, the high sensitivity of PCR can lead to false positive results associated with contamination of samples, which is a serious disadvantage of the method.

Real-time PCR and quantitative BLV analysis

RT-PCR (real-time PCR) is based on the quantitative detection of a fluorescent signal, which increases in proportion to the amount of the PCR product. Therefore, the real-time PCR result can be recorded in dynamics during the reaction, at the current time. The modern real-time PCR test system contains a pair of primers and an additional TagMan probe complementary to the inner fragment region. Two molecules are covalently connected to the probe: a fluorescent label ("reporter") at the 5-end, which emits fluorescence, and a fluorescence quencher ("quencher") at the 3-end, which extinguishes the fluorescence of the fluorescent molecule. During the reaction, Taq polymerase, moving along the matrix, reaches the probe and cleaves it. At the same time, the distance between the fluorescent label and the fluorescence attenuator increases, and the fluorescence is no longer extinguished. Contamination of samples and false positive reactions are excluded when using real-time PCR technology. Real-Time PCR allows for not only qualitative, but also quantitative analysis, and eliminates the appearance of false positive results [21].

The most common variant of real-time PCR using fluorescent DNA probes, primarily linear destructible samples (Taq-manassay, TaqMan). Several diagnostic kits have been developed and used in Russia and abroad to detect BLV based on the PCR-RV method [2, 6, 10, 11].

Research Methods

For molecular genetic studies, genomic DNA samples of black-and-white cows isolated from the peripheral blood of animals were used.

Blood was taken from cattle in a number of farms in the Bryansk

Table 1: Diagnosis of bovine leukemia provirus in groups of animals of different ages and health conditions.

№	Animal Group	Number of animals	Number of virus carriers	
			Heads	%
Calves up to 6 months ago				
1	Group 1	25	2	8,0%
2	Group 2	70	4	5,7%
3	Group 3	50	-	0%
	TOTAL	145	6	4,1%
Adult cows				
4	Group 4	90	3	3,3%
5	Group 5	50	18	36,0%
6	Group 6	49	13	26,5%
	TOTAL	189	34	18,0%
RID+ Cows				
7	Group 7	85	45	52,9%
Animals with hematological manifestations of leukemia				
8	Group 8	14	14	100%

region of Russia, in total, various studies were conducted on 433 samples of cattle genomic DNA.

The following groups of animals were formed for the analysis:

1) Adult animals – 189 heads;

2) Calves up to 6 months old – 145 heads;

3) RID + animals – 85 heads;

4) Animals with hematological manifestations of leukemia – 14 heads.

A standard technique was used to isolate genomic DNA [2].

Using agarose gel electrophoresis, it was shown that the samples obtained have a sufficiently high concentration of DNA aligned with the samples.

In the method of detecting BLV provirus during Real-Time PCR, three primers and two fluorescent destructible samples were used to synthesize two fragments of oligonucleotides – 220 bp and 178 bp. Amplification was performed using a programmable thermostat with the detection of the ANK-32 fluorescent signal. Electrophoresis in 2% agarose gel was performed to determine the length of the synthesized fragments and to prove their specificity.

Figure 1 shows the synthesis of an oligonucleotide 220 bp long, the samples differ in different contents of the DNA matrix (dilution by 32 and 1024 times, respectively). The amplification efficiency with the used oligonucleotide set is on average 0.87 or 87%, this value characterizes the technique as highly effective.

Results

Application of the PCR-RV method for screening cattle for BLV virus transmission

Cattle were screened for provirus DNA on 433 samples of animal genomic DNA from seven cattle farms. In total, 4 groups of animals of different age groups and different health conditions were formed.: 1) calves up to 6 months old; 2) adult dairy cows; 3) RID+ dairy cows; 4) animals with hematological manifestations of leukemia.

As an example, Figure 2 shows the result of the determination of the BLV provirus in calf blood samples.

The results of the PCR-RV analysis of BLV in animals of all 4 groups are presented in the table 1.

As follows from Table 1, when analyzing BLV virus transmission by PCR-RV method of animals of 4 groups from farms of the Bryansk region, 99 heads of cattle were identified as carriers of the leukemia virus.

The data on the distribution of virus carriers by groups are shown in Figure 3.

As shown in Figure 3, the spread of the virus among young animals (groups 1-3) is significantly lower than in other groups and ranges from 0 to 8%, due to the presence of a placental barrier blocking the vertical transfer of the virus from infected cows to newborn calves.

A comparison of virus transmission in groups of calves of dairy cows in groups 4-6 shows a significant difference – from 8% to 36%, which reflects the different stages of infection of animals with the

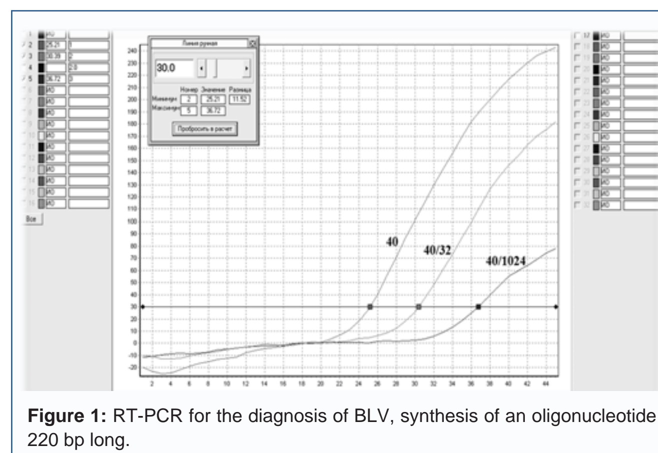

Figure 1: RT-PCR for the diagnosis of BLV, synthesis of an oligonucleotide 220 bp long.

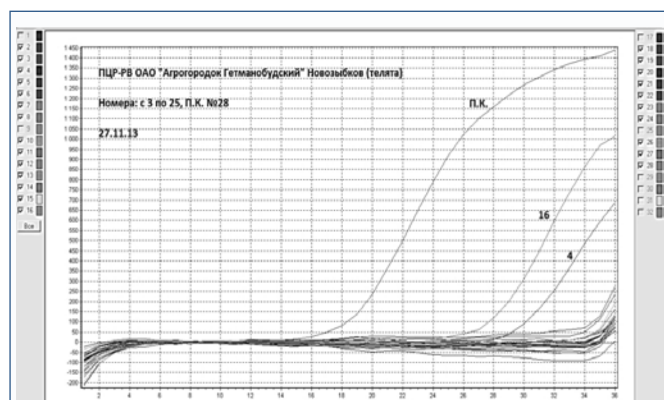

Figure 2: Determination of BLV provirus in calf blood samples by RT-PCR.

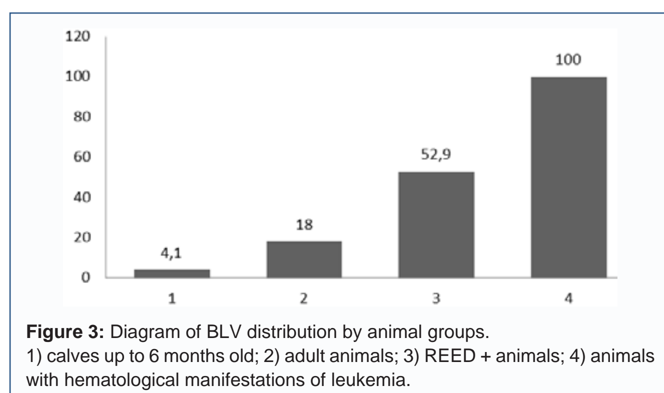

Figure 3: Diagram of BLV distribution by animal groups.
1) calves up to 6 months old; 2) adult animals; 3) REED + animals; 4) animals with hematological manifestations of leukemia.

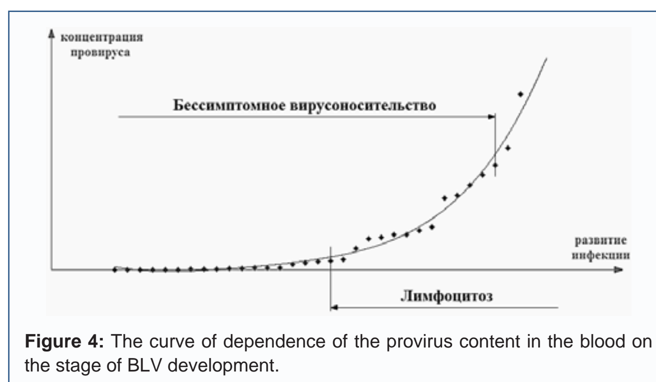

Figure 4: The curve of dependence of the provirus content in the blood on the stage of BLV development.

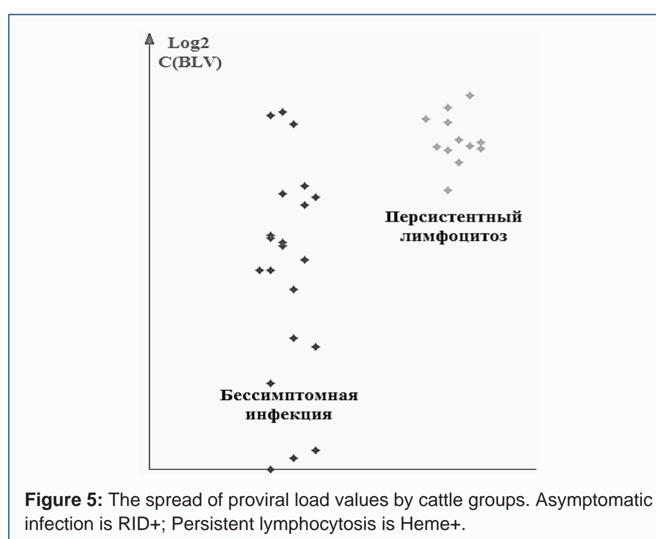

Figure 5: The spread of proviral load values by cattle groups. Asymptomatic infection is RID+; Persistent lymphocytosis is Heme+.

virus.

Among RID+ cattle, half of the animals did not have leukemia provirus, which indicates either the genetic resistance of animals to the persistence of the virus in peripheral blood during the development of protective antibodies to BLV, or that in the first years of the incubation period, the virus was not isolated. At the same time, with clinical signs of leukemia, all sick cows are virus carriers and virus carriers.

Thus, the data obtained indicate the possibility of using the developed RT-PCR method to study the prevalence of BLV in cattle herds. The use of RT-PCR is of particular importance due to the need for early molecular and genetic diagnosis of the virus in order to subsequently cull infected calves in order to form a healthy herd of repair young animals and fattening groups.

A small part of the screening tests performed were quantitative in nature, and were conducted to study the dynamics of the infectious process in the selected groups of animals – healthy (RID-), asymptomatic virus carriers (RID+, Hem-), patients with hematological changes (Hem+).

Quantitative analysis was performed among infected (20 RID+ animals) and sick (12 Heme+ animals) livestock. It was found that the content of proviral DNA in the blood of sick animals is on average 45 times higher than that of virus carriers. However, this is not a general pattern for each group of cattle, since the variation in the values of the initial provirus content is significant, especially for the RID+ cattle sample. The results are visualized in Figures 4 and 5.

Thus, the use of the RT-PCR method for the detection of BLV makes it possible to detect infected cattle with high accuracy from the moment of calving, as well as to conduct a quantitative analysis of BLV accumulation.

Discussion

The high prevalence of BLV in cow herds in livestock farms around the world requires the development of effective methods to combat leukemia.

Characteristics of the RT-PCR method for the analysis of BLV proviral DNA

We have developed a molecular genetic method for analyzing BLV proviral DNA using RT-PCR, for which we have received a patent [13]. This proviral DNA analysis is highly sensitive, highly specific, high-performance, high-speed and cheap.

During amplification, oligonucleotides with lengths of 178 and 220 bp are synthesized. According to the proposed temperature-time protocol, the analysis duration is on average 1.5 hours. At 40 cycles, RT-PCR has a sensitivity of 50-100 copies of provirus per sample, and several copies of proviral DNA per sample at 45 cycles. The method is 90% effective.

The main advantages of the PCR-RV technique should also include the exclusion of contamination and, as a result, false positive samples, the possibility of quantitative diagnosis, and the possibility of using PCR-RV to detect provirus in young animals in the presence of colostral antibodies.

Conducting mass DNA analysis of different groups of cows by RT-PCR method

DNA analysis of different groups of cows by RT-PCR confirmed the correlation between the age of the animals and the level of virus transmission: among young animals (groups 1-3), provirus was detected much less frequently than in the group of healthy dairy cows – 0-8% and 8%-36%, respectively. This confirms that the placental barrier blocks the vertical transmission of the virus during pregnancy. An increase in virus transmission to 25% in young animals 6 months before the first application of RID confirms that the main route of virus transmission is horizontal transmission of BLV between animals in the herd.

In the RID+ group of animals BLV provirus was detected in 52.9% of animals, that is, 47.1% of cows did not have provirus, possibly they have genetic resistance to the persistence of the virus in peripheral blood and sufficient production of protective antibodies when infected with BLV.

In the group of sick cows with hematological manifestations of the disease, BLV was detected in all animals, that is, they are virus carriers and virus separators.

A quantitative analysis of the dynamics of virus accumulation conducted among infected (RID+) and diseased (Hem+) animals showed that the content of proviral DNA in the blood of sick animals is on average 45 times higher than that of virus carriers (Figures 4, 5). However, this is not a common pattern within each group of cows, since the variation of values of the initial provirus content are significant, especially for the RID+ cattle sample.

The conducted studies indicate the applicability of the RT-PCR method for studying the prevalence of BLV in cow herds, including for the early molecular genetic diagnosis of provirus in newborn calves and young animals. Early detection of virus carriers among newborn calves and young animals (up to 1-3%) allows them to be promptly culled, form a healthy repair herd and prevent horizontal transmission of the virus.

Thus, we propose a method of accelerated recovery of cattle from BLV, which includes the following set of measures:

1. Early diagnosis of BLV provirus by the molecular genetic RT-PCR method using blood samples from newborn calves and young animals;

2. Identification and timely culling of virus–carrying calves; formation of a BLV-free repair stock of cattle. At the same time, a virus-free repair herd can be formed immediately after calving (1-3 days), when the level of infection of animals with the BLV virus can be 1-3%, which prevents horizontal transmission of the virus.

3. Breeding of virus-free repair young animals, keeping calves in a clean area, drinking milk from REED cows and controlling isolation from potential virus-carrying animals.

Thus, the proposed method of accelerated recovery of cattle from the bovine leukemia virus is characterized by the complexity and feasibility of measures, has a pronounced economic result, which generally leads to a successful result.

References

1. Apalkin V.A, Gulyukin M.I, Petrov N.I. Bovine leukemia (diagnosis and health measures). St. Petersburg: Petrolaser, 2005. 87 p.

2. Akhmedov R.B, Nam I. Yan. G, Zayakin V.V. Quantitative method of analysis of bovine leukemia provirus using polymerase chain reaction in real time. Bulletin of Agroindustrial Complex of Stavropol. 2020; No. 4 (40): 35-41

3. Deren W, Szewczyk-Sadowska A, Rulka J. The eradication of enzootic bovine leucosis in a large farm population. Pol J Vet Sci. 2003; № 6: 12-14.

4. Eaves F.W, J.B. Molloy C.K. Dimmock L.E. Eaves A field evaluation of the polymerase chain reaction procedure for the detection of bovine leukaemia virus proviral DNA in cattle. Vet. Microbiol. 1994; V. 39: 313-321.

5. Heinecke N, Tortora J, Martinez HA, Gonzalez-Fernandez VD, Ramirez H. Detection and genotyping of bovine leukemia virus in Mexican cattle. Arch Virol. 2017; Jul 8. doi: 10.1007/s00705-017-3477-z.

6. Jimba M, Takeshima S.N, Murakami H, Kohara J, Kobayashi N, Matsuhashi T, et al. BLV-CoCoMo-qPCR: a useful tool for evaluating bovine leukemia virus infection status. BMC Vet. Res. 2012; 8: doi: 10.1186/1746-6148-8-167.

7. Johnson R, Kaneene J.B. Bovine leukemia virus. P.1. Descriptive epidemiology, clinical manifestation, and diagnostic tests. Continuing education article No 12. The Compendium, Noth American Edition. Food Animal. 1991; V.13, №2: 315-327.

8. Juliarena M.A, Poli M, Sala L, Ceriani C, Gutierrez S, Dolcini G, Rodríguez E.M, Mariño B, Rodríguez-Dubra C., Esteban E.N. Association of BLV infection profiles with alleles of the BoLA-DRB3.2 gene. Animal Genetics. 2008; V. 39. № 4: 432–438.

9. Khusainov R.F. Colostral immunity and intrauterine infection of calves with leukemia virus from maternal cows with varying degrees of severity of the infectious process: abstract of the dissertation of the cand. Biology: 16.00.03. Ufa, 2009: 22 p.

10. Kosovsky G.Y., Klimov E.A, Goryunov D.V, Sivolapova A.B. Patent 2521330 RF, IPC C2 G01N. A method for detecting the bovine leukemia virus by nucleotide sequences of conserved regions of the viral genome; Application 15.06.2012; published 20.12.2013.

11. Kuckleburg G.C, Chase C.C, Nelson E.A, Marras S.A, Dammen M.A, ChristopherHennings J. Detection of bovine leukemia virus in blood and milk by nested and realtime polymerase chain reactions. J. Vet. Diagn. Invest. 2003; V.15. №1: 72-76.

12. Mammerickx M, Portetelle D, Nys J, Burny A. Rapid detection of bovine leuksfemia virus infection in a large cattle population with an ELISA performed on pooled sera grouped by herd. Zbl. Vet. Med. B.- 1985; Bd.32: 601-608.

13. Nam I. Ya, Akhmedov R.B, Zayakin V.V. Patent 2747192 "Sets of oligonucleotides for quantitative determination of bovine leukemia provirus". 29.04.2021. The priority date is 07.07.2020.

14. Ruzina M.N. Analysis of the BoLA-DRB3 gene polymorphism in connection with the genetic resistance of cattle to virus-carrying leukemia. Moscow. 2012. – 142 p.

15. Sagata N, Yasunaga T, Ogawa Y, Tsuzuku-Kawamura J, Ikawa Y. Bovine leukemia virus: unique structural features of its long terminal repeats and its evolutionary relationship to human T-cell leukemia virus. Proc. Natl. Acad. Sci. U.S.A. 1984; 81: 4741–474510.1073/pnas.81.15.4741.

16. Saiki R.K, Gelfand D.H, Stoffel S, Higuchi R. Primer-directed enzymatic amplification of DNA with a thermostadle DNA polymerase. Science; 1988. Vol. 239: 487.

17. Simonyan G.A. Development and improvement of health-improving anti-leukemia measures. Veterinary medicine. 2007; No. 7: 3-7.

18. Simonyan G.A. Modern methods of control of bovine leukemia and elimination of economic damage. Veterinary medicine. 2011; No. 9: 3-8.

19. Sourvinos G, Tsatsanis C, Spandidos D.A. Mechanisms of retrovirus-induced oncogenesis. Folia Biol (Praha). 2000; 46 (6): 226-32. Review.

PMID: 11140855.

20. Suh G. H, Lee J.C, Lee C.Y. Establishment of a bovine leukemia virus-free dairy herd in Korea. J Vet Sci. 2005; Sep. № 6(3): 227-230.

21. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. Biotechniques. 2008; 44: 619–626.

22. Zhangkai J. Cheng, Jing Shan. 2019 Novel coronavirus: Where we are and what we know (англ.). 2020 Apr; 48(2): 155-163. doi: 10.1007/s15010-020-01401-y. Epub 2020 Feb 18.