



## **Improving Diagnostic and Eliminating Techniques of Bovine Leukemia in the Russian Federation**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Leukemia occupies a leading place in the structure of infectious diseases of cattle, as it causes significant economic damage to livestock and poses a serious social danger. The dynamics of infection with the bovine leukemia virus (LV) have been studied in the Krasnodar Territory for ten years. A decrease in the level of infection of animals with the bovine leukemia virus was noted at livestock enterprises in the public sector and in breeding farms. In the individual sector, due to an

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imperfect animal insurance system, farmers try to keep IDT+ animals on their farms. Some progress in reducing the infection of cattle with the leukemia virus in the Krasnodar Territory has been achieved through a whole range of measures. This includes the publication of relevant regulations governing the solution of this problem by the state and regional authorities, the coordination of recreational activities by scientists of the Russian Academy of Sciences and employees of the Department of Veterinary Medicine, propaganda work among the population and heads of livestock enterprises, and the introduction by veterinary specialists of modern methods of the early diagnosis of bovine leukemia virus. The order of the Ministry of Agriculture No. 156 dated 03/24/2021 became a timely document to reduce the time of the recovery of cattle from leukemia in Russia. It significantly tightens the requirements to the terms of recovery, regulates the introduction of new methods of the early diagnosis of leukemia. As a part of the implementation of the research topic, the authors proposed a new diagnostic kit for the detection of bovine LV provirus in finished dairy products, which makes it possible to accelerate the identification of infected animals in farms that supply products to dairy plants, and in general, accelerates the elimination of leukemia in Russia.

*Keywords: Leukemia; bovine; epizootic situation; laboratory diagnostics; IDT; ELISA; PCR; diagnostic kit; the order of the Ministry of Agriculture No. 156; health improvement.*

## 1. INTRODUCTION

Bovine leukemia is currently one of the most pressing problems in veterinary medicine. Bovine leukemias are diagnosed almost in all countries of the world [1,2,3]. They are most widely distributed in the United States, several Central European countries, Denmark, Sweden, the Middle East, and some African countries [4]. The researches to find effective methods of diagnosing and recovering from this disease is constantly being carried out by leading scientists around the world [5,6,7].

The danger of leukemia is that it proceeds for a long time without clinical signs [28-14]. It is manifested only in the terminal stages by lymphocytosis and tumor formations of hematopoietic organs and tissues. The causative agent of the cattle is an RNA-containing virus [2,15]. The source of the pathogen is animals with myeloblastosis. The leukemia virus can be transmitted prenatally and postnatally under natural conditions. The virus is transmitted vertically from mother to offspring via the transplacental route. Horizontal transmission of the virus occurs through raw or unpasteurized milk or contact [16,17]. In more than 90 % of cases, the virus is transmitted with infected lymphocytes. In the present time, direct evidence on the relationship between bovine leukemia and human infection has not been established. However, it has been proven that the bovine leukemia virus (LV) is similar to the human T-cell leukemia virus [18]. The milk of cows with leukemia is unsafe for human health due to the content of carcinogenic metabolites in it. In this regard, according to veterinary regulations, such

milk is not allowed for human consumption. An important role of the milk of cows infected with leukemia has also been established in the spread of infection among calves [19,20]. Back in 2003 G.C. Buehring et al. showed the presence of not only antibodies to bovine LV in human blood but also the presence of a provirus in them, which the authors attribute to direct or indirect contact between humans and infected animals, as well as to their products [15].

The complexity of the fight against bovine leukemia lies in the lack of effective and simple methods of diagnosing bovine LV, as well as significant economic losses due to the culling of sick and infected animals [21,4]. The relevance of the topic for animal husbandry in Russia allowed us to set the following tasks:

- To study the spread of bovine leukemia in livestock enterprises of various forms of ownership;
- To develop a new diagnostic PCR system to determine the provirus of the leukemia virus in finished dairy products;
- To develop recommendations for livestock enterprises to reduce the time of recovery of livestock from leukemia;
- To improve methods of quality control of finished dairy products at processing enterprises in Russia.

## 2. MATERIALS AND METHODS

The epizootological monitoring was used in the course of the research, including epizootological observation, analysis, and forecast of the

epizootic process. Data collection and analytics on the spread of the leukemia virus in livestock enterprises of the Krasnodar Territory of various forms of ownership were carried out [22,23]. Comparative historical analysis was carried out for the last 10 years. Their reliable epizootological analysis makes it possible to predict the development and manifestation of the epizootic process at any of its stages. Clinical, pathomorphological, and laboratory methods were used to study the spread of bovine leukemia virus, serological such as IDT (immunodiffusion test) and ELISA (enzyme-linked immunosorbent assay), as well as the method of molecular genetics diagnostics as PCR (polymerase chain reaction) [24-27]. The results were subjected to statistical processing [28].

The object of the study was cattle of various ages. An analysis of the state of animals on leukemia was carried out in a large agricultural region with developed dairy farming. According to the Office of the Federal State Statistics Service on the Krasnodar Territory and the Republic of Adygea (Krasnodarstat), on April 1, 2021, the number of cattle in the Krasnodar Territory is 551.4 thousand heads. To analyze the epizootic situation, we used the data of veterinary reporting of the Department of Veterinary Medicine of the Krasnodar Territory and the Kropotkin Regional Veterinary Laboratory on farms of the Krasnodar Territory of all forms of ownership. To identify virus-carrying animals, early diagnostic methods were used – enzyme-linked immunosorbent assay (ELISA) and PCR, thanks to which the virus carrier in animals can be determined from the first days after birth (ELISA) and from the 20th day of the calf's life (PCR). Evaluation of the effectiveness of methods of the recovery of cattle from leukemia was carried out taking into account the timing and frequency of the research of animals, the initial degree of infection of the herds and the number of livestock, the production orientation of the farms, and their economic condition. Seropositive animals were examined annually by the hematological method with simultaneous control of the blood plasma in IDT [29,25,30]. The reliability and objectivity of the results of the study were compared with the dynamics of indicators of the intensity and extensiveness of the epizootic process of the bovine leukemia virus in certain areas of the Krasnodar Territory [31]. The key to the successful implementation of recreational activities is constant monitoring and timely diagnosis of bovine leukemia [29,21,7].

The basis of the development of a complex of health-improving measures in the region was taken from the developments of scientists from the Ural RVI “Ural system of health-improving anti-leukemia measures” [6,21]. Farms, settlements, and administrative territories (districts, oblasts, krais, republics) were considered to be satisfactory in terms of leukemia, in which animals with leukemia are not detected during routine diagnostic studies, as well as during slaughter of animals at a meat processing plant [8].

Animal welfare control is carried out by veterinary specialists of farms, the state veterinary service, and meat processing plants based on a set of indicators for TSE at meat processing plants and planned serological and hematological studies on leukemia.

The primary diagnosis in a leukemia-free household is established based on positive results of serological and hematological or pathomorphological testing.

### 3. RESULTS AND DISCUSSION

In our country, the occurrence of bovine leukemia is associated with the importation of breeding animals from Europe in the 40s of the last century [1,2]. In Russia, the problem of the spread of leukemia is relevant to this day, since agricultural enterprises in most regions of the country are unfavorable to this disease [1,4]. The International Association for Comparative Research on Leukemia and Related Diseases must unite scientific research areas of scientists all over the world. There are scientific works about some features of the combined course of these diseases and the need for further scientific research in this direction [5,3].

It has been proven that the milk of cows with leukemia is unsafe for human health due to the content of carcinogenic metabolites in it. In addition, as noted above, the important role of milk from leukemia-infected cows in the spread of infection among calves has been established. Foreign researchers have presented evidence that bovine LV may be associated with the development of breast cancer [9].

The epizootic situation on bovine leukemia in the Krasnodar Territory from 2009 to 2018 was tense [17] since, during the analyzed period, bovine LV was again registered in 32 municipalities of the region, in which 177 unfavorable areas were

**Table 1. The results of laboratory tests on bovine leukemia in breeding farms for 2012-2021 (according to SBI KT "Kropotkin Regional Veterinary Laboratory")**

Year	Hematology				IDT			
	Total research (voice)	Animals with leukemia (heads)	%	Animals of suspicious leukemia (heads)	%	Total research. (voice)	IDT(+) (heads)	%
2012	33 075	305	0.9	1 044	3.2	128 367	3 657	2.9
2013	24 617	274	1.1	675	2.7	114 401	3 692	3.2
2014	11 950	48	0.4	322	2.6	95 255	1 559	1.6
2015	9 501	31	0.3	229	2.4	105 805	1 459	1.4
2016	9 501	34	0.3	570	6.0	119 269	1 265	1.0
2017	8 565	10	0.1	399	4.6	127 342	1 131	0.9
2018	6 858	0	0.0	562	8.1	134 923	1 528	1.1
2019	2 828	0	0.0	9	0.3	135 076	651	0.4
2020	6 754	0	0.0	760	11.3	124 432	469	0.38
2021	8231	0	0.0	345	4.2	129 581	482	0.37

identified. The largest number of unfavorable areas was registered in 2013. Significant forces of veterinarians were brought in to change the situation.

Positive dynamics are observed after analyzing the results of the study of cattle on leukemia in the breeding farms of the Krasnodar Territory for 10 years, (Table 1). As can be seen from the data in the table, no animals with leukemia have been identified over the past three years. The percentage of IDT (+) in breeding farms decreased up to 0.37 %. Out of 129,581 heads, 482 tested positive. Such animals are immediately isolated and, following the plans of recreational activities, are slaughtered.

The situation with the improvement of cattle from leukemia in the individual sector is worse. Although there are practically no animals with leukemia left (5 animals out of 29,382 tested), the percentage of animals suspicious of the disease is high. Thus, in 2021 it increased to 7.8 %, compared with 6.5 % in 2020. The total number of infected animals in the public sector for 2021 was 6.6 %, in 2020 it was 8.9 %. Thus, the overall infection of cattle with the leukemia virus in 2021 was decreased by 2.3 %. Difficulties with the recovery of farms from leukemia are since the compensation that farmers receive for the delivery of sick, suspicious, and IDT+ animals do not cover losses from their culling. The fear of economic losses prevails over the social responsibility for the supply of low-quality livestock products. At the end of 2021, 47 disadvantaged points remain in the Krasnodar Territory, from which 33 are in the public sector and 14 in the private. For the reporting period of 2021, restrictions were lifted

from three unfavorable areas. Including two in the public sector and one disadvantaged area in the private.

Currently, under the guidance of scientists from the Russian Academy of Sciences (Academician I.M. Donnik), scientists from the Faculty of Veterinary Medicine of FSFEI HE "Kuban State Agrarian University named after I.T. Trubilin", employees of the State Budgetary Institution KT "Kropotkinskaya Regional Veterinary Laboratory" with the support of the Department of Veterinary Medicine of the Krasnodar Territory, together with the employees of the FSBEI FPE "St. Petersburg Institute of Management and Food Technologies" the research is being carried out on the topic: "Development of a new molecular genetic method of laboratory diagnosis of bovine leukemia virus to improve the livestock enterprises of the Russian Federation". This work is carried out on the order of the Department of Science and Technology Policy and Education of the Ministry of Agriculture of Russia. As a part of the implementation of this program, together with the employees of "Vetfactor" LLC, a "Kit for detecting DNA of the provirus of bovine leukemia in finished food products by DNA amplification with fluorescence detection in real-time" was developed and tested. The new kit makes it possible to specifically amplify a fragment of the bovine leukemia virus genome and internal positive control DNA in a multiplex polymerase chain reaction. The kit has now passed production tests in veterinary laboratories and has shown good results. The absence of non-specific reactions of the kit components against other pathogens was shown: influenza A virus, the causative agent of Aujeszky's disease, bovine diarrhea virus, infectious bovine rhinotracheitis virus, bovine parainfluenza-3

virus, bovine coronavirus, *Brucella abortus*; *Brucella ovis*; *Brucella canis*; *Pasteurella multocida*; *Leptospira interrogans*; *Listeria monocytogenes*; *Mycobacterium avium*; *Escherichia coli*; *Campylobacter jejuni*; *Campylobacter fetus*; *Clostridium perfringens*; *Salmonella dublin*; *Staphylococcus aureus*; *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Mycobacterium tuberculosis*, *Mycobacterium paratuberculosis*.

It becomes possible to determine the bovine VL provirus genome in finished dairy products using a new diagnostic kit, which will make it possible to carry out health improvement activities much more efficiently by identifying infected animals in the suppliers' herds. All this corresponds to the strategic line of the government of the Russian Federation and the Ministry of Agriculture of the Russian Federation (Order No. 156 dated March 24, 2021), aimed at the speedy recovery of the Russian cattle population from leukemia and achieving food security [8,18].

#### 4. CONCLUSION

The problem of bovine leukemia in terms of biological, epizootic-epidemic, socio-economic, and some other factors is still an urgent problem for science and practice in modern conditions. Further research work on the study of the features and patterns of bovine VL, the disclosure of the mechanisms of their manifestation will allow us to successfully carry out appropriate preventive and health-improving measures, and will significantly complement the theory of infectious and epizootic processes of infectious diseases. Leukemias and other hemoblastoses, because of their significance, are still the object of a close study of the biological, veterinary, and medical sciences. The acuteness of the problem of hemoblastoses is given by the similar development and clinical and morphological manifestation of these diseases in animals, humans, and even birds. This necessitates the further study of this contagious pathology to more successfully carry out anti-leukemic measures. The fact that the regions with a high percentage of affected cattle have a higher incidence of human leukemia indirectly underlines the danger of the bovine leukemia virus (BLV) to humans. The Order No. 156 of the Ministry of Agriculture, which entered into force on September 1, 2021, and is dedicated to tightening measures to combat this insidious chronic disease in Russia and other countries of the Eurasian community, can be considered

timely. It legislates the use of new, more advanced methods for the early diagnosis of bovine leukemia. The modern legislative framework, prepared by the Ministry of Agriculture will be the catalyst that will significantly accelerate the recovery of livestock enterprises in all regions of Russia from bovine leukemia.

Order of the Ministry of Agriculture of the Russian Federation of March 24, 2021 No. 156 "On the approval of the Veterinary rules of the implementation of preventive, diagnostic, restrictive and other measures, the establishment and cancellation of quarantine and other restrictions aimed at preventing of the spread and eliminating of foci of leukemia in cattle" [8] will intensify work on the speedy recovery of livestock enterprises from bovine leukemia. The new diagnostic kit proposed by us for the determination of bovine LV provirus in finished dairy products will speed up the identification of infected animals in the farms of suppliers, and, consequently, accelerate the elimination of leukemia in all regions of Russia.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

All authors hereby declare that the research was conducted in strict accordance with ethical principles established by the European Convention on the protection of the Vertebrata used for experimental and other scientific purposes (adopted in Strasbourg on March 18, 1986, and confirmed in Strasbourg on June 15, 2006) and the ethical standards laid down in the 1964 Declaration of Helsinki.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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