Biotechnological Communication

Bovine Anti-leukemic Measures for Improving Live-Stock Farms in Belgorod and Kemerovo Regions of Russia

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ABSTRACT

Bovine leukemia remains one of the most urgent viral diseases in veterinary medicine, and potentially dangerous for humans. The strategy of combating it is aimed at improving the existing measures and full recovery of agricultural enterprises. The quality of animal products is a priority in the field of food safety. The aim of the research was to develop effective antileukemic measures for the improvement of livestock farms in the Belgorod and Kemerovo regions. The proposed antileukemic measures are to increase the frequency of serological studies from 6-th months to the 2-3th months among animals in areas with poor leukemia in cattle, as well as to increase the sensitivity of immunodiffusion test (AGID) due to highly centrifigation of the tested samples and an increase in the temperature of incubation samples close to the physiological norm for animals. The improved technique of staging an immunodiffusion reaction (AGID) allows detecting an average of 12% more infected animals with BLV in comparison with the approved method of staging a serological reaction AGID. Intensive introduction of PCR diagnostics to identify the causative agent of bovine leukemia in young calves, after the neonatal age period, will allow detecting early infection of animals and adjusting the program of antileukemia measures in disadvantaged farms. The introduction of PCR diagnostics in calves in the postnatal period of development in permanently dysfunctional livestock farms will contribute to the recovery of young animals from cattle leukemia in dysfunctional farms. The proposed antileukemia measures for the improvement of livestock farms in the Belgorod and Kemerovo regions made it possible to develop effective preventive measures for disadvantaged farms, improving the epizootic situation in the regions. So, in 2021, it was possible to completely improve the permanently dysfunctional economy of the LLC « Pobeda» in the Belgorod region.

KEY WORDS: ANTILEUKEMIC MEASURES DIAGNOSTICS, BOVINE LEUKEMIA VIRUS, ENZOOTIC BOVINE LEUKOSIS, POLYMERASE-CHAIN-REACTION, REHABILITATION OF DISADVANTAGED FARMS.

INTRODUCTION

Protection of animal and human health is the main task of antiepizootic and antiepidemic services in different countries of the world. Bovine leukemia (EBL) is one of the most pressing infectious viral diseases in animals. This is a slow-moving infectious disease of a tumor nature, which causes enormous economic and economic damage to livestock farms, and also poses a potential medical and social danger to humans (Frie et al. 2016; Stepanova 2016; Sviridenko 2017; Mishchenko et al. 2018; Murakami et al. 2019; Donnik et al. 2021; Kovalenko et al. 2020; Kovalenko et al. 2021). Modern approaches to recovery from bovine leukemia require modernization in laboratory diagnostics of this disease, thereby preventing the spread of viral infection (DeBrogniez et al. 2015; Gulyukin et al. 2019). The effectiveness of antileukemia measures depends on the improvement of laboratory diagnostics methods and earlier detection of infected animals with the bovine leukemia virus during the incubation period, as well as strict observance of veterinary and sanitary measures to form sustainable welfare for bovine leukemia (Gulyukin et al. 2015; Donnik et al. 2015; Valikhov 2018; Donnik et al. 2021).

In recent years, there has been a directed process to improve the health of dysfunctional farms for bovine leukemia through constant laboratory monitoring and isolation of identified infected individuals (Donnik 2011). The basic test for detecting infected EBL cows is the immune

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diffusion reaction (AGID), and the final diagnosis is made after hematological studies (Donnik et al. 2013). Modern diagnostics of bovine leukemia is based on the identification of the causative agent of the disease, which requires molecular genetic studies (PCR). The molecular genetic method is highly sensitive and reliable, which opens up new opportunities for the study of this disease. The aim of the research was to develop effective antileukemic measures for the improvement of livestock farms in the Belgorod and Kemerovo regions (Mishchenko et al. 2018; Murakami et al. 2019; Donnik et al. 2021).

MATERIAL AND METHODS

The studies were performed in the Belgorod State Agricultural University named after V.Ya. Gorin "and dairy farms of the Belgorod region – LLC «MMF Nezhegol»(dairy farm No. 1), JSC «Orlik» (dairy farm No. 2), CJSC «Voskhod» (dairy farm No. 3) and LLC «Pobeda» (dairy farm No. 4) in the period 2020-2021 years (Donnik et al. 2021). The object of research is blood serum and stabilized blood of cattle from farms with poor leukemia in cattle. Blood samples for research were taken from the caudal vein with disposable instruments into sterile tubes. Diagnosis of animals with leukemia in cattle is carried out by studying the hematological parameters of the blood of animals that previously reacted positively in the reaction of immunodiffusion. As an additional method to the main diagnostic tests carried out - serological and hematological studies, PCR diagnostics using the test-system «Leukemia» was used on young cows after the 14th day of rearing up to six months of age, obtained from AGID (+) cows in LLC «Pobeda» (dairy farm No. 4). The laboratory studies of cow samples were carried out according to unified methods (Order of the Ministry of Agriculture and Food of the Russian Federation of May 11, 1999 N 359) and the protocol of serological studies (Donnik et al. 2021).

Using an automatic hematological analyzer "URIT-3020", the leukocyte formula and the calculation of the content of certain types of leukocytes were determined. Microspopic assessment of stained smears according to Leishman was determined on Labor microscopes, 60 × /0.75 with a built-in camera; for molecular genetic studies (PCR), the Leukemia test system was used according to the instructions for its use. For statistical data processing, mathematical models were used on a personal computer (Murakami et al. 2019).

RESULTS AND DISCUSSION

The antileukemia measures carried out on the territory of the Belgorod and Kemerovo regions began, first of all, with epizootological monitoring and accounting of all stationary dysfunctional farms, while laboratory diagnostics always plays a special role. the developed improved formulation of the immunodiffusion reaction (AGID) and an increase in the frequency of sampling from the studied animals helped to more effectively identify infected animals and carry out comprehensive health measures on the disadvantaged territory of farms. The proposed antileukon measures are to increase the frequency of serological studies carried out per year among animals in unfavorable areas for bovine leukemia and the time of the beginning of specific studies, in the postnatal period of the calf. Improvement in the diagnosis of bovine leukemia consists in increasing the sensitivity of the AGID due to the highly centrifugation of the tested samples and increasing the temperature of the temperature control of the samples *in vitro*, bringing it closer to the physiological norm for animals in vivo (Kovalenko et al. 2021).

In the experimental part of the work, during the development of an improved laboratory technique for the formulation of the AGID reaction, 1251,0 samples of cattle blood serum from were taken from a leukemia-dysfunctional farm - LLC «Pobeda» (dairy farm No. 4). They were investigated using various methods of high-speed centrifugation and temperature conditions close to the physiological parameters of the animal in order to identify the maximum number of additional AGID (+) positive bovine heads that previously gave a negative reaction of immune diffusion in the standard formulation of AGID (Kovalenko et al. 2020; Kovalenko et al. 2021; Donnik et al. 2021). The proposed antileukon measures are in the Improved method of staging the immunodiffusion reaction, which makes it possible to identify, on average, 12% more infected BLV animals in comparison with the approved method of staging a serological reaction. The use of the proposed method for improving the diagnosis of leukemia in cattle made it possible to identify cows infected with leukemia at earlier stages of the incubation period, which are in the initial period of the production of antileukemic antibodies, which made it possible to reduce the time spent by these animals in the herd and contributed to the acceleration of health measures without significant material and time costs (Donnik et al. 2021).

In the complex of antileukemic measures with a goal the recovery of dysfunctional farms, we proposed to reduce the frequency of serological tests from 6 to 3 months, and, if possible, laboratory tests can be carried out more often, based on specific cases. Since a six-month study period can contribute to the re-infection of AGID -negative animals with infected individuals in the early stages of the development of the leukemia process, in which antibodies to BLV were not previously detected in the immunodiffusion reaction (AGID) (Kovalenko et al. 2020; Donnik et al. 2021).

The antileukemic measures used consisted in the fact that in addition to the existing objective informational tests to determine the infection (using the immunodiffusion reaction) and the morbidity of animals from among the infected, an automatic hematological analyzer can also be used as an additional tool, the data of which make it possible to determine the severity of the course leukemic process. So, in dairy farm No. 4 antileukemic measures were worked out to improve the economy as following: step-by-step identification of sick animals; determination of the area of prevalence of the pathogen; study of the level of cow leukemia virus infection on the farm; establishment of ways and mechanisms of transmission of the bovine leukemia virus in a particular farm; formation of schemes for isolated rearing of young animals; development of

technological charts for the repair of livestock by uninfected young animals. So, on the dairy farm No.2at the beginning of 2020 (01/01/2020) the level of infection was 31.7% (there were 3526 animals in total), and in June (01/06/2020) no positively responding animals were detected in the AGID, thanks to the implementation of isolating preventive veterinary and sanitary measures aimed at improving the animal population (Fig. 1) (Kovalenko et al. 2020; Donnik et al. 2021).

Figure 1: The results of the use of anti-leukemia measures in improving the economy in Dairy farm No.2

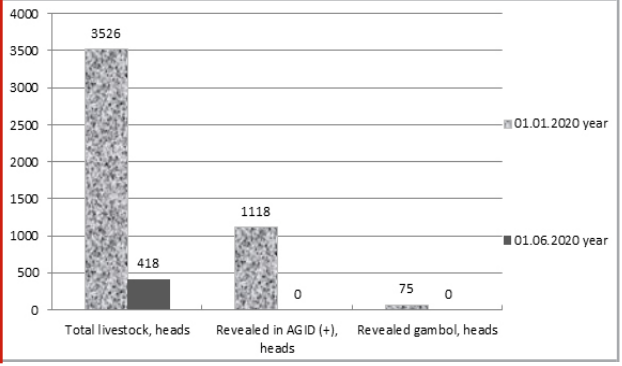
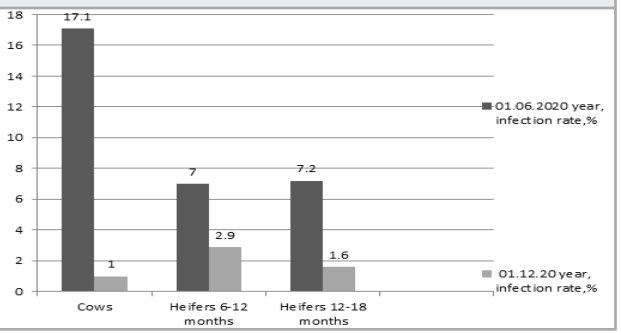


Figure 2: The level of infection of animals by BLV in Dairy farm No.4

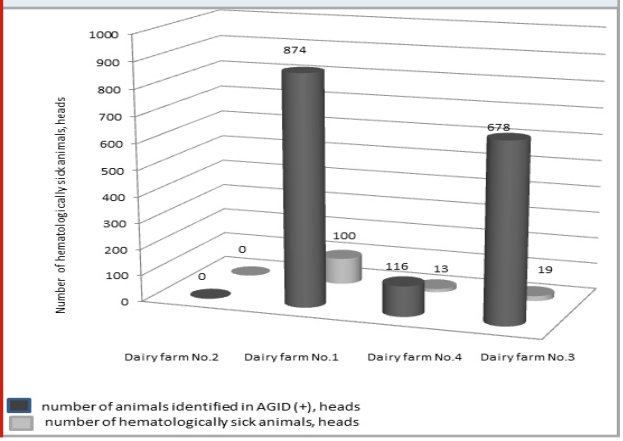


In January 2020, in four dysfunctional livestock farms (EBL) of the Belgorod region with a population of 6605 heads, a positive reaction in the AGID, 3343 heads, which were 50.6% of the total number of livestock, and among them, hematologically sick individuals of 219 headswere identified (6.55%). Since the livestock farm of «Dairy farm No.4» was unfavorable for leukemia in cattle for many years, where the level of infection of the livestock with BLV infection significantly exceeded 30% infection of the herd, it was decided to use this farm as a model. According to the improved method of early detection of animals infected with the leukemia virus in cattle, blood sampling for serological studies should be carried out not every six months, but every 2-3 months. So, in Dairy farm No.4, by the end of 2020 the aplication of the developed technique for early diagnosis of leukemia made it possible to increase the detectability of infected livestock in the immunodiffusion reaction (AGID), in comparison with the beginning of the study period, 01.01.2020, from 1668 to 1741 heads, or 6.35% (Donnik et al. 2021).

At the same time, there were only 22 animals with hematologically sick cattle leukemia at the end of 2020, which in percentage terms was 1.26%. Thus, as a result of laboratory studies of samples from infected cows, a direct dependence of the concentration of the desired antibodies in the tested sera and the influence of the temperature factor of incubation on the absolute binding of antibodies to antigens was established under conditions of the immunodiffusion reaction more physiologically close to in vivo. This provided an increase in the sensitivity of ACID and made it possible to detect, on average, 20.25% more animals infected with the leukemia virus, compared with the staging of a standard AGID (Kovalenko et al. 2021; Donnik et al. 2021).

The result of PCR studies carried out in the beginning 2020, the presence of BLV was found in 16 samples (11.11%) from 114 calves (blood samples). Taking into account these results, this test was used in studies conducted in May – June 2020. As a result, in May, the presence of BLV was detected in 14 out of 50 blood samples from calves aged 15 days to 3 months (28% of the total number of examined individuals), in June - in 2 out of 36 blood samples of animals from 15 days to 1.5 months (5.5%). In general, in 2020, the presence of the BLV was detected in 32 calves under 6 months of age out of 230 individuals born from AGID-positive cows, which amounted to 13.9%. All animals, in whose blood BLV proviral DNA was detected, were removed from the general herd and fed in a separate slaughter house after gaining the appropriate weight (Donnik et al. 2021).

Figure 3: Detection rate of hematologically sick individuals in relation to the total number of cattle leukemia virus infected



Antileukemia measures, consisting in the application of the developed diagnosis of EBL, contributed to the recovery from leukemia infection of the cattle of Dairy farm No.2, and in two other previously dysfunctional leukemia farms of Dairy farm No.3 and Dairy farm No.4 of the Belgorod region to reduce the level of infection by November 2020 from 56.4% and 84.1% to 41.4% and 11.5%, respectively. The developed approach to the diagnosis of leukemia in cattle during health-improving measures made it possible to completely recover from leukemic infection the number of cattle of Dairy farm No.2 and in the other two previously unsuccessful farms of Dairy farm No.3 and Dairy farm

No.4 of the Belgorod region to reduce the level of infection by November 2020 from 56.4% and 84.1% to 41.4% and 11.5%, respectively (Donnik et al. 2021).

Additional serological studies (AGID) carried out within six months, 01.06.-01.12.2020, in the dysfunctional farm of Dairy farm No.4showed that the level of infection in cows of all sex and age groups, as well as heifers 6 ... 12 and 12 ... 18 months, decreased to 1%, respectively, 2.9% and 1.6% (Fig. 2). In general, in this farm, the infection rate of animals with bovine leukemia decreased from 84.1% at the beginning of 2020 to 1.6% at the end of 2020, which indicates the effectiveness of the health-improving measures taken.

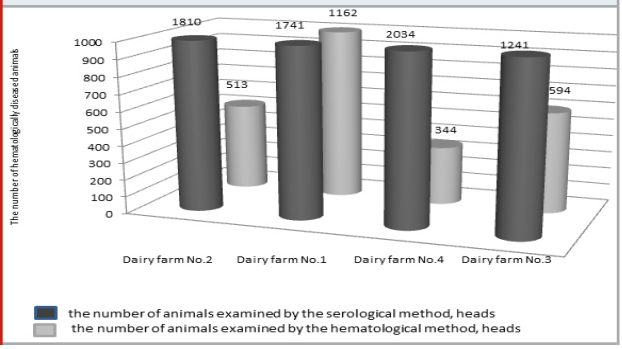
The results of a serological study of the blood serum of the Dairy farm No.4 livestock (828 heads), carried out twice in February and April 2021, showed that there were no animals that responded positively in the AGID. Additional PCR studies of 21 samples from calves from 15 days of age in February 2021 also did not reveal the genomic material of the causative agent of bovine leukemia. In addition, to study the severity of the pathological process, we used an automatic hematology analyzer URIT-3020, which allows, in addition to quantitative study of the content of the leukocyte pool (the number of leukocytes), to detect the content of granulocytes (basophils, eosinophils, neutrophils), lymphocytes and monocytes.

According to the results of the conducted hematological studies of 2613 heads of cattle during 2020, the presence of 132 hematological individuals in permanently dysfunctional farms was established. So, in LLC "Nezhegol" 100 hematologicallysick cows were identified among 874 RID (+) heads, in LLC "Pobeda" 13 hematologicallyheads among - 116 heads;at Voskhod CJSC there are 19 hematologically sick players among - 678 heads, which in percentage terms was 11.4%, respectively;11.2%;2.8% (fig. 3). So, in Dairy farm No.1 there were identified 100 hematologically sick among 874 AGID (+) heads, in Dairy farm No.4 - 13 hematologically sick among 116 heads; at Dairy farm No.3 there are - 19 hematologically sick players among -678 heads, which in percentage terms amounted to 11.4%, respectively; 11.2%; 2.8% (Fig. 3). Using the standard technique for determining the number of leukocytes using the Goryaev camera, it was found that among 1668 serologically positive animals in dysfunctional breeding farms of the Belgorod region, only 132 hematologically patients were identified, which amounted to 7.9% (Table 1).

Table 1. Statistical data of hematological studies in permanently dysfunctional farms in the Belgorod region

Name farms	Investigated serological method, heads	Investigated by hematological method, heads	Revealed hematologically sick animals, heads	Rented hematologically sick animals, heads	Remaining hematologically sick animals on 30.11.20г., heads
Dairy farm No.1	1741	1162	100	78	22
Dairy farm No.2	1810	513	0	0	0
Dairy farm No.3	1241	594	19	39	0
Dairy farm No.4	2034	344	13	16	0
TOTAL	6826	2613	132	133	22

Figure 4: The ratio of the number of hematologically studied animals to the total number of serological studies



Based on the "leukemia key", the status of cattle in stationary dysfunctional farms was determined: up to 12 thousand / μl (healthy animals), and over 12 thousand / μl infected and sick animals (from 12 to 20 thousand / μl - the initial stage of the hematological stage of leukemia; from 20 to 30 thousand / μl - the middle stage of the hematological stage of leukemia; over 30 thousand / μl - the transition to the tumor stage of leukemia. In general, for all unfavorable stationary farms of the Belgorod region 2613 heads were examined by hematological method among seropositive individuals in AGID, and 6826 animals were subjected to serological research, which amounted to 38.2%; at the same time, 132 hematologically sick heads were identified and 133 (100%) were handed over for slaughter, and at the end of this year there were 22 heads left (Table 1).

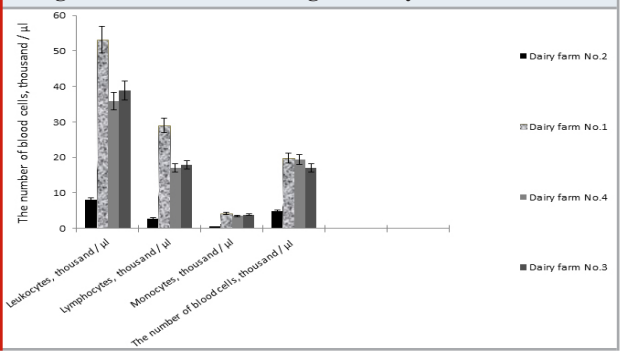
It is also worth noting that the ratio of the number of hematologically studied animals to the total number of serological tests (AGID) in stationary dysfunctional livestock agricultural enterprises of the Belgorod region - Dairy farm No.2; Dairy farm No.1; Dairy farm No.4 and Dairy farm No.3 were as follows: 513 heads by 1810 heads (28.3%); 1162 heads to 1741 heads (66.7%); 344 heads

by 2034 heads (16.91%) and 594 heads to 1241 heads. (47.56). (fig. 4).

Assessing the effectiveness of standard hematological studies, it should be noted that the excretion rate of hematologically sick animals among the total number of seropositive animals in stationary cattle leukemic cattle breeding enterprises of the region ranges from 0.05% to 1.53%. The standard method for determining the number of leukocytes in the studied blood samples does not reveal the depth of development of persistent leukocytosis in infected animals. For this purpose, hematological studies were additionally carried out to identify the formed elements of the leukocyte pool (granulocytes, monocytes and lymphocytes) in cattle blood samples using an automatic hematological analyzer URIT-3020.

Thus, it was found that the greatest damage to the hematopoietic system of animals was in the farm of Dairy farm No.1 with a total infection rate of more than 80%: the average number of leukocytes in 1 μl of tested blood samples was 53.1 ± 2.3 thousand / μl, lymphocytes 29.1 ± 1.7 thousand / μl, monocytes 4.2 ± 0.4 thousand / μl, granulocytes 19.8 ± 2.0 thousand / μl (Fig. 5).

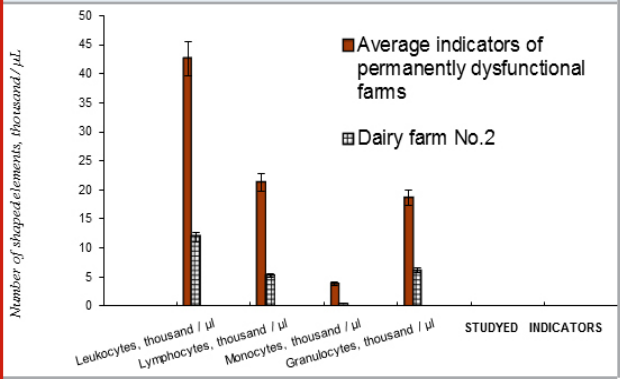
Figure 5: Results of hematological studies at hematologically sick livestock of livestock enterprises in the Belgorod region using an automatic hematological analyzer URIT-3020.



Further, according to the degree of infection (more than 40%) and the development of the leukemic process of the cattle population, the stationary dysfunctional economy of Dairy farm No.3, where the indicators of the content of leukocytes, lymphocytes, monocytes and granulocytes on average among sick animals were 38.9 ± 3.0, respectively. thousand / μl, 17.9 ± 3.3 thousand / μl, 3.9 ± 0.7 thousand / μl and 17.1 ± 1.3 thousand / μl (Fig. 5). In Dairy farm No.4, the indicators of the content of leukocytes, monocytes, lymphocytes and granulocytes on average among sick animals were, respectively, 35.8 ± 3.1 thousand / μl, 17.1 ± 2.3 thousand / μl, 3.5 ± 0.8 thousand / μl and 19.4 ± 1.5 thousand / μl (Fig. 5).

In general, for all stationary dysfunctional farms among infected animals BLV, an average of leukocytes, lymphocytes, monocytes, and granulocytes, respectively, 42.6 ± 2.9 / thousand / μl, 21.3 ± 1.9 thousand / μl, 8 ± 0.5 thousand / μl, 18.7 ± 1.8 thousand / μl (Fig. 5). In Dairy farm No.2 (free from leukemic infection at the end of the year) no hematologically sick individuals were found, therefore the indicators of the content of leukocytes, monocytes, lymphocytes and granulocytes were practically close to the reference ones and amounted to 11.9 ± 1.3 thousand / μL, respectively, 5, 3 ± 1.4 thousand / μl, 0.5 ± 0.9 thousand / μl, and 6.1 ± 0.8 thousand / μl. (Fig. 6).

Figure 6: Average hematological indicators in stationary dysfunctional livestock farms



The data of hematological parameters for the leukocyte pool indicate the severity of the development of the leukemic process and make it possible, in addition to the existing tests, to characterize the qualitative changes in the pools of the agranulocyte and granulocyte series. The unified method for counting the number of formed elements - a hematological test using a Goryaev chamber makes it possible to determine quantitative indicators of the content of leukocytes in 1 μl, but does not make it possible to determine the differentiated composition of blood leukocytes (granulocytes and agranulocytes), which is especially important for determining the stage of development of the leukemic process during complex health-improving antileukemic measures. Studies conducted over several decades of the spread of leukemia in cattle, led to the conclusion that not all breeds are equally susceptible to the leukemia virus.

The most susceptible were black-and-white breeds with an influx of Dutch, German and Danish blood (Forlettia et al. 2013). The bovine lymphocyte antigen (BoLA) system is the main constituent of the bovine histocompatibility complex. The genes of the histocompatibility complex encode highly polymorphic cell surface molecules, which are antigenic peptides of T cells (Forlettia et al. 2013), thereby playing a decisive role in the immune response to foreign agents. In cattle, three DRB genes in the composition of BoLA were identified in the histocompatibility complex (Valikhov 2018; Murakami et al. 2019; Kovalenko et al. 2020).

However, only the DRB3 gene is the main pronounced locus, since DRB1 is a pseudogene, and the DRB2 gene, as shown by Northern blot analysis, is not expressed in bovine leukocytes or is expressed at a very low level. The DRB3 gene (class II gene), which encodes the b-chain of the histocompatibility complex, is the only functional DRB gene actively transcribed in cattle. Exon 2 of the BoLA DRB3 gene has a high level of polymorphism and

is represented by more than 100 different alleles described to date (Úsuga-Monroy et al. 2016). When introducing innovative methods of molecular genetics into the breeding process of improving domestic populations of dairy cattle, it is necessary, first of all, to investigate the molecular genetic basis that determines the emergence and formation of certain characteristics of an organism, including susceptibility and resistance to infectious diseases, in particular to the bovine leukemia virus. In this case, it is necessary to take into account the specialization in the productivity of certain populations of livestock, since the incidence of leukemia among dairy cattle is significantly higher than that of beef cattle.

Therefore, within the framework of the research of scientists of the Kuzbass Agricultural Academy, the task was to determine the resistance to the leukemia virus in black-and-white cattle by the Bola-DRB3 gene and to identify animals that carry not only generally recognized alleles of resistance to leukemia, but also alleles that are specific genetic markers of resistance to leukemia virus. The object of research was the animals of the Kemerovo population of the black-and-white breed. Studied 187 animals: 122 of them are sick and 65 healthy. In studies, the greatest polymorphism was noted for the alleles of the BoLa-DRB3 gene, attributed by the majority of researchers to neutral - 47 variants were identified. In total, the frequency of these alleles was 43.6%. Generally neutral allelic variants are less than 5% of the frequency each. At the same time, it should be noted that the most common alleles are 0601 - 7.8% and 1801 - 7.2%. The analysis of the obtained results of the study showed that the frequency of occurrence of alleles 1801 and 0601 in the groups of patients with leukemia and healthy animals differed significantly. The frequency of the 0601 allele in healthy animals is 11.7 times higher ($P < 0.01$) than in the group of sick animals. The frequency of the 1801 allele in healthy animals is 8.3 times higher ($P < 0.05$) than in the group of sick animals (Murakami et al. 2019; Kovalenko et al. 2020).

Since the data on the number of leukocytes in AGID (-) negative animals have physiological normative indicators, it can be said with confidence that the data on the quantitative ratio of leukocyte elements (lymphocytes, monocytes and granulocytes) could be used when working out the technique in the case of stable reliable results to characterize the course of the leukemia process, using it as a test for early diagnosis of infected animals BLV and at the final stage of health-improving anti-leukemia measures. The method of early diagnosis of bovine leukemia developed by us made it possible to speed up the process of rehabilitation of permanently disadvantaged farms of LLC «POBEDA» during the year and so thanks the full implementation of the plan of preventive health improvement (anti-leukemia) measures developed on the territory of the Belgorod region in 2021 year. For the implementation of health-improving measures, the following stages were proposed for identifying animals with leukemia:

- conducting epizootological studies and dividing the herd into infected AGID (+) and conditionally healthy AGID (-);
- isolation of infected animals indoors;
- placement of livestock infected with the leukemia virus on separately located farms, which contain exclusively infected animals, in accordance with the instructions for combating bovine leukemia, taking into account their routes of movement both on the territory of the corresponding departments and inside livestock areas, separate milking, separate service personnel and the use of technical means (Kovalenko et al. 2020).

The developed antileukemia measures help to effectively carry out the process of rehabilitation of disadvantaged livestock farms by increasing the frequency of serological studies and increasing the sensitivity of the immunodiffusion reaction AGID. The use of the proposed methods helps to detect infected cattle in the early incubation period and prevent further spread of the pathogen through the separation of animals in the system of general veterinary and sanitary antiepizootic measures. This reduces the time spent by infected animals in the herd, contributing to the acceleration of antileukemia health measures without significant material and time costs. An additional effect is provided by the use of molecular genetic tests that allow identifying the causative agent of BLV in young calves in the post-neonatal period of their development (Kovalenko et al. 2020).

CONCLUSION

The findings of the present study proposed antileukemia measures for the improvement of livestock farms in the Belgorod and Kemerovo regions made it possible to develop effective preventive measures for disadvantaged farms, improving the epizootic situation in these regions. The use of the developed antileukemia measures made it possible in 2021 year to completely improve the number of animals from BLV in one of the inpatient dysfunctional livestock farms of the Belgorod region.

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