Prevention and rehabilitation of dairy herd from cattle leukemia

T Zubova¹, V Pleshkov¹, N Chalova¹, O Prokhorov¹, O Smolovskaia¹, E Izhmulkina¹, and A Mironov¹
¹Kemerovo State Agricultural Institute, Markovtseva str., 5, Kemerovo, 650056, Russia
E-mail: suta54@mail.ru

Abstract. The main diagnostic aids and response methods for the cattle leukemia virus that are currently used are represented in the article. The wide spread of the disease is caused by a fairly easy transmission of the virus, the lack of early diagnostic methods, vaccination and treatment. The problem of the cattle leukemia virus is very acute and is an urgent issue in animal husbandry, which requires an urgent solution.

1. Introduction
The main supplier of livestock products in the Russian Federation is dairy and beef cattle breeding, which is able to ensure food security of the population regardless of other states. At the same time, the main indicators of livestock production include an increase in the volume of high-quality agricultural products and decreasing its production cost.

Creating the herd consisting of a highly productive healthy livestock is a prerequisite for the intensive development of animal husbandry. One of the diseases that impedes development and causes enormous damage to livestock breeding is the wide spread of the cattle leukemia virus. This disease causes livestock enterprises great economic damage. Along with a significant decrease in productive indicators, premature culling of cows and stud bulls is carried out, as well as forced slaughter of animals on commercial and pedigree farms, which entails a breeding limitation and the loss of the gene pool of highly productive animals [1-5].

Enzootic bovine leukosis is a chronic viral disease characterized by a malignant organ lesion of the lymphoid and hematopoietic systems. It is included in the B list of cattle diseases of the World Organization for Animal Health (OIE) and is a disease of a mandatory declaration [6-8].

This disease is recorded in almost all countries of the world. The exceptions are some countries of Western Europe and a number of other states, where it was eliminated only by the complete elimination of infected animals. Despite such exceptional measures, disease cases are still detected in the cattle populations of these countries [7, 9]. The disease is most widespread in the USA, Canada, Japan, and a number of countries in Eastern Europe. Among the tested animals, 83.9% of dairy herds in the USA turned out to be seropositive for the leukemia virus, for Canada this indicator was 89%, 84% for Argentina, 68% for Japan; in Brazil and South America the rate of cattle leukemia virus infection is 50%, in Central Asia it is about 20% [10-17].

The expansion problem of cattle leukemia virus in Russia is acute and is an issue requiring urgent solutions. The state of livestock farms in most country regions regarding this disease is unsuccessful.
The response measures that are used in some foreign countries and which include the complete one-time replacement of the herd cannot be implemented in our country for a number of reasons.

According to the Veterinary Medicine Department of the Agriculture Ministry of the Russian Federation [18] “... Cattle leukemia in 2017 was registered in 67 regions of the Russian Federation (in 68 regions in 2016).

The epizootic situation for cattle leukemia has improved in Kursk, Oryol, Tambov, Kaliningrad, Novgorod, Kirov, Nizhny Novgorod, Ulyanovsk, Sverdlovsk, Irkutsk regions, the Udmurt Republic, Krasnoyarsk Territory. In these members, the number of dysfunctional spots and animals infected with leukemia decreased.

The epizootic situation regarding cattle leukemia in the Novosibirsk region remains difficult, there are 222 dysfunctional points, 127 points in Chelyabinsk region, 109 in Kaluga region, 100 in Krasnodar Territory, 92 in Moscow region, 87 in Kurgan region, 74 in Tverskoy region, 66 in Samara region, 62 in Republic of Tatarstan, 62 in Primorsky Territory, 58 in Kemerovo Region, 53 in Tyumen, 53 in Penza and other entities ...

High incidence of cattle leukemia is determined by a fairly easy virus transfer, the lack of early diagnosis aids, vaccination and treatment of this disease.

Diagnosis should ensure the rapid detection of infected animals and at the same time be quite simple and effective. There are various methods for detecting cattle leukemia virus carriers, which include the immunodiffusion test (IDT), enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), hematological, clinical, pathomorphological studies and biological tests [19, 20].

Serological (IDT and ELISA) and hematological research methods are mainly used in Russia for the diagnosis of cattle leukemia. But such commonly used diagnostic methods are not able to detect all infected animals due to the absence or low level of antibodies. Serological tests can also show mixed results in the early stages of animal disease, and false positives can occur. Moreover, such methods do not provide absolute detection of infected animals, as calves remain outside the planned studies up to six months of age, due to the lack of detectable antibody formation in them.

In this regard, using the molecular polymerase chain reaction (PCR) genetic method for recognizing infection is quite promising. In recent years, this method has been successfully used to detect carriers of retroviral infections to detect proviral DNA integrated into the host genome. The PCR method has maximum sensitivity and high specificity while conducting research. These factors contribute to the ability to detect the virus in the test sample 1-2 weeks after infection. The polymerase chain reaction method is also suitable for diagnostic work with calves older than 15 days [21-25].

In Russia today, the solution to the problem of cattle leukemia is based on the "Rules for the Prevention and Control of Cattle Leukemia", approved by Agriculture and Food Ministry order of the Russian Federation No. 359 of 05.11.1999.

The key motive of the Rules is the regulation of diagnostic serological and hematological researches, removal (with virus-bearing up to 10%) and isolated content (with infection up to 30%) of infected animals and hematological diagnosis (with infection more than 30%) and slaughter of sick cows. According to the Rules, the prevention and control of leukemia consists in culling sick animals and isolating infected animals, followed by their gradual replacement with healthy ones. The whole herd of cattle should be checked for leukemia from six months of age according to the IDT reaction, and hematological studies should be conducted from two years of age. Serological tests for IDT are performed at the age of 6, 12, 16, 18 months. Hematologic studies of an adult livestock (IDT positive) are performed every six months.

Leukemia is diagnosed based on the blood tests results on the immunodiffusion test (IDT), which is strictly specific. If the positive immunodiffusion reaction is registered, the animal is separated into a room apart from a healthy livestock and hematological blood tests are performed. The number of leukocytes, poorly differentiated cells, and also polymorphic atypical cells is detected in peripheral blood after carrying out hematological blood tests. If hematological changes in the blood of animals are detected, they are considered to be sick with leukemia [21].
The fundamental factors in carrying out preventive work and resisting leukemia in the Kemerovo region, depending on the degree of damage to the livestock on farms, are the replacement or slaughter of infected cow groups, the quarterly isolation and urgent slaughter of virus carriers and of HEM sick animals.

Measures to combat cattle leukemia in the Kemerovo region are carried out in accordance with the following:
- Plan for diagnostic studies, veterinary prophylactic and anti-epizootic measures in farms of all ownership forms;
- The rules for the prevention and control of cattle leukemia, approved by Order of the Ministry of Agriculture and Food of the Russian Federation of 05.11.1999 No. 359;
- Guidelines for the diagnosis of cattle leukemia, approved by the head of the Veterinary Department of the Ministry of Agriculture of the Russian Federation on 08.23.2000 No. 13-7-2/2130.

Farm improvement, regardless of ownership form, is carried out on the document basis of the Kemerovo Region Veterinary Administration “On the declaration of trouble and imposing restrictions” No. 247 dated 09.14.2015, No. 11 dated 02.05.2016, No. 12 dated 05.02.2016, No. 8 dated 02.03.2017, and developed individual plans.

According to statistics and analysis of farms on the well-being of leukemia, the disease is asymptomatic for most animals infected with the cattle leukemia virus (about 70%), while approximately 30% of infected animals develop a mild form of the disease - persistent lymphocytosis. Further development of the disease, which transfers into lethal lymphosarcoma, occurs within 5% of cases for infected animals [26-28].

Cattle leukemia virus infection is carried out in two ways: horizontal (postnatal) and vertical (prenatal).

Currently, the main way is considered to be the horizontal transmission of the cattle leukemia virus. In case of horizontal transmission of the virus, circumstances favorable for the spread of the disease include violating the rules for veterinary measures and non-compliance with the requirements for the isolation of infected and virus-free animals.

Despite the danger of the disease and its fairly easy transmission between animals, it was determined that more than 80% of calves from infected cows are born free of the leukemia virus. This is due to the fact that only in 10-15% of cases, the virus overcomes the intraplacental barrier of the mother, infecting the fetus. A versatile and qualified approach to the problem of the disease will allow to completely update the dysfunctional herd after several years without purchasing the expensive breeding animals, but by growing a sufficient number of virus-free pedigree animals [29-31].

Having information about the nature of the disease and the ways of its transmission, it can be stated that one of the more acceptable and permissible methods of farm improvement is the identification of sick and infected animals during diagnostic studies and their further exclusion from the herd. Therefore, strict adherence to "Rules for the Prevention and Control of Cattle Leukemia", approved on 05.11.1999; to measures of controlling cattle leukemia conducted in the Kemerovo region in accordance with the annual plans for diagnostic studies, veterinary preventive and anti-epizootic measures in farms of all forms of ownership; to individual plans of farms with all ownership forms and the timely implementation of the whole anti-leukemia measure complex in farms, it can create a favorable situation for the incidence of livestock, allowing receiving products and save offspring for further introduction into the herd without a single liquidation of the livestock.

The prerequisite for the elimination of the cattle leukemia virus is the active participation of both the veterinary service and animal owners. At the same time, organizational, economic and administrative measures for the prevention and control of leukemia play a crucial role.

The aim of the study was to improve the herd of cattle from the leukemia virus through a series of measures. The studies were conducted in the peasant farm of an individual entrepreneur V.D. Zinchenko of the Kemerovo region on cattle of black and motley breed. Measures for controlling the cattle leukemia virus were to identify sick and infected animals during routine diagnostic tests and their further exclusion from the herd. The results of the study showed a positive trend in the release of
the herd from leukemia virus. A separate content of IDT + animals, isolated rearing of pedigree animals (from the number of offspring from sick and infected cows) for their subsequent replacement and further inclusion in a healthy herd give a significant reduction in the number of virus carriers and sick animals.

2. Materials and methods

The object of the study was healthy, seropositive (IDT +) and leukemic cows of black-motley breed, kept in the peasant farm of an individual entrepreneur V.D. Zinchenko, Kemerovo region.

Studies conducted on cows of black-motley breed showed a positive dynamics of the stopping the leukemia virus among the herd. The method of isolated rearing of pedigree animals from the sick and infected cows’ offspring was used for their gradual replacement when conducting rehabilitation measures [29].

Implementation of rehabilitation activities was carried out in accordance with the developed “Plan of measures for the prevention and control of cattle leukemia for 2014-2019 in the peasant farm of an individual entrepreneur Zinchenko VD” [32], which presumes the implementation of established organizational and economic measures.

The plan section of the organizational and economic measures includes actions establishing the order of sale, delivery for slaughter, pasture, placement on pastures and all other movements and regrouping of animals; their clear numbering and labeling; picking different farms with IDT-positive and IDT-negative animals; the formation of individual groups and herds consisting of pedigree animals; keep heifers out of the main herd of cows; on a farm with RID-negative animals, only artificial insemination and other work can be carry out.

The plan section of veterinary measures establishes the procedures and terms for hematological and serological studies of animals; organization of calving of healthy and infected cows; organization of feeding and rearing of young animals and disinfection of livestock buildings and slaughter areas.

3. Research results

Due to the separate keeping of the dysfunctional group of animals on a separate farm and the timely execution of diagnostic tests in order to detect infected and sick animals, it was possible to achieve some success in improving the herd.

The dysfunctional group of animals with leukemia is housed at farm No. 1 and consists of cows, pedigree animals, stirks aged 6-16 months and calves up to 6 months old. IDT - negative young stock is selected from this farm for transfer to a healthy herd to another farm.

The rehabilitated herd is located on the farm No. 2, and this group includes IDT - negative cows, pedigree animals and stirks older than 6 months of age. It is a leukemia-free farm that is constantly replenished with seronegative stirks after their first diagnostics at 6 months of age.

In general, positive dynamics of combating the leukemia virus is noted according to the family farm IP Zinchenko V.D.

| Table 1. | The degree of damage to the livestock by years (farm No. 1). |
|----------|--------------------------------------------------------------|
| Indicator| Year | 2016 | 2017 | 2018 |
| Total amount of animal units at the farm | | 1012 | 867 | 860 |
| IDT | Tested animal units | | 2799 | 2280 | 1435 |
| | Positively responding animal units | | 199 | 183 | 64 |
| | % | | 7 | 8 | 4.45 |
| Hematologic studies | Tested animal units | | 760 | 613 | 805 |
| | Positively responding animal units | | 16 | 19 | 7 |
| | % | | 2 | 3 | 0.86 |
So, 199 IDT + responding animal units were registered at a farm No. 1 (Table 1) in 2016 (7% of the studied population), in 2017 it was 183 animal units (8% of the studied population), then in 2018 it was only 64 animal units (4.45% of the population). Hematological studies during these years revealed 16 (2% of the studied livestock) positively responding animal units in 2016, 19 (3% of the studied livestock) animal units in 2017, and only 7 (0.86% of the studied livestock) in 2018.

**Table 2.** The degree of damage to the livestock by years (farm No. 2).

| Indicator                  | 2016  | 2017  | 2018  |
|----------------------------|-------|-------|-------|
| Total amount of animal units at the farm | 750   | 640   | 624   |
| IDT                        |       |       |       |
| Tested animal units        | 2874  | 1976  | 1315  |
| Positively responding animal units | 48    | 14    | 18    |
| %                         | 1     | 0.7   | 1.36  |
| Hematologic studies        |       |       |       |
| Tested animal units        | 10    | 7     | 16    |
| Positively responding animal units | 0     | 0     | 0     |
| %                         | 0     | 0     | 0     |

There is an ambiguous situation regarding livestock restoration after the leukemia virus in the convalescent herd at the farm No. 2 (Table 2), since 48 IDT + responding animal units (1% of the studied livestock) were registered in 2016, 14 animal units in 2017 (0.7% of the studied population), but 18 animal units were identified (1.36% of the studied population) in 2018. Hematological studies of the livestock for 2016-2018 did not reveal a single positively responding animal.

4. Conclusions

Thus, following certain rules and requirements for the prevention and control of cattle leukemia virus, including the systematic work of introducing a healthy livestock and the separation of IDT +, results in a significant reduction in newly detected virus carriers and sick animals.

The measures for rehabilitating the livestock population with leukemia virus used in the economy show positive dynamics, but there are also certain difficulties. It is necessary to have free premises for the isolation of animals, special operating personnel working only with a livestock free of the leukemia virus, as well as equipment and other means of keeping and servicing animals. These factors carry an additional burden on livestock farming and economic indicators of livestock production.

Difficulties arising during the rehabilitation process of livestock farms make it necessary to find modern scientific ways to solve the rehabilitation problem of livestock with leukemia. Therefore, the development and improvement of measures to fight leukemia in cattle is a significant and relevant topic in animal husbandry.

The article was prepared as part of an agreement with the Russian Ministry of Education and Science No. 05.607.21.0208 “Development of genomic editing technology for reproduction of high-value breeding dairy cattle husbandry resistant to leukemia virus” unique identifier of the agreement RFMEFI60718X0208

**References**

[1] Simonian G 2011 *Veterinariia* 9 pp 3-8

[2] Gendzhiev A, Chimidova N, Gendzhieva O 2014 *Biology and Medicine* 6 4 BM-055-14

[3] Zubova T, Pleshkov V, Mironov A 2018 *Siberian Journal of Life Sciences and Agriculture* 10 5 pp 119–131 DOI: 10.12731/wsd-2018-5-119-131

[4] Benitez O, Roberts J, Norby B, Bartlett P, Grooms D 2019 *Theriogenology* 126 pp 187-190

[5] Tsutsui T, Kobayashi S, Hayama Y, Yamamoto T Preventive 2016 *Veterinary Medicine* 124 pp 96-101

[6] Vsesimnainia organizatsiia zdravookhraneniia zhivotnykh http://www.oie.int

[7] Berry D, Bermingham M, Good M, More S 2011 *Irish Veterinary Journal* 64 p 5

[8] Prokhorov O, Pleshkov V, Zubova T, Mironov A, N Solomina Y 2018 *International Journal of*
Mechanical Engineering & Technology (IJMET) 9 13 pp 796-802

[9] Informatsionno Analiticheskii Tsentr RSKhN FGBU «VNIIZZh» http://www.fsvps.ru/fsvps-docs/ru/iac/foreign/2013/dec/partners.pdf

[10] Guliukin M, Kozyreva N, Ivanova L 2015 Voprosy virusologii 60 5 pp 32-37

[11] Zavershinskaia O, Komissarov S, Zavershinskii A 2013 Vestnik TGU 18 1 pp 447-450

[12] Krasnikova E, Larionova O, Krasnikov A, Kazieva G 2018 Vopr. pitaniia 87 4 pp 48-55

[13] Rudakova O 2010 Analiz sovremennykh metodov diagnostiki i veterinarno-sanitarnaiya ekspertiza misa pri leikoze krupnogo rogatogo skota Dissertatsiia na soiskanie uchenoi stepeni kandidata biologicheskikh nauk (Moscow) p 141

[14] Stregnii B, Shapovalova O, Gorbatenko S, Korneikov A, Gorzheev V 2013 Veterinarna meditsina 97 pp 242-255 http://www.jvm.kharkov.ua/sbornik/97/3_97.pdf

[15] Bartlett P, Norby B, Byrem T, Parmelee A, Erskine R 2013 Journal of Dairy Science 96 3 pp 1591-1597

[16] Yang Y, Chu S, Shang S, Yang Z, Wang C 2019 Journal of Dairy Science 102 4 pp 3469-3473

[17] Kuczewski A, Hogeveen H, Orsel K, Wolf R, Meer F 2019 Journal of Dairy Science 102 3 pp 2578-2592

[18] Pismo Departamenta veterinarii Ministerstva selskogo khoziaistva RF № 25/735 ot 30 marta 2018 g http://agroportal2.garant.ru:81/SESSION/PILOT/main.htm

[19] Vinogradova I, Gladyr E, Kovaliuk N, Petropavlovskii M, Donnik I, Ernst L, Zinoveva N 2011 Dostizheniia nauki i tekhniki APK 10 pp 34-37

[20] Ponomarenko D, Abakin S, Kalashnikova E 2011 Veterinariia selskokhoziaistvennykh zhivotnykh 9 pp 20-24

[21] Bobkova G, Shamaro P, Prudnikova T 2011 Vestnik Brianskoi GSKhA pp 42-48

[22] Kosovskii G, Glazko V, Andreichenko I, Kovalchuk S, Glazko T 2016 Selskokhoziaistvennaia biologia 51 4 pp 475-482

[23] Kriukov V, Shalimova O, Drushliak N, Pikunova A 2012 Vestnik OrelGAU Nauchnoe obespechenie zhivotnovodstva 1 pp 62-68

[24] Saushkin N, Samsonova J, Osipov A, Kondakov S 2019 Journal of Virological Methods 263 pp 101-104

[25] Petersen M, Alvarez I, Trono K, Jaworski J 2018 Journal of Dairy Science 101 7 pp 6366-6374

[26] Guliukin M, Baranov I, Ivanova L 2016 Veterinariia i kormlenie 4 pp 4-41

[27] Ivanov O, Ivanova O 2015 Farm Animals 1 pp 22-24

[28] Nieto Farias M, Souza F, Lendez P, Martinez-Cuesta L, Dolcini G 2018 Veterinary Immunology and Immunopathology 206 pp 41-48

[29] Novoseltsev G, Karabaktian V, Simonian G, Repnikova N 2019 Sait Departamenta veterinarii Krasnodarskogo kraia http://www.kubanvet.ru/journal_n1_20113.html

[30] Frie M, Coussens P 2015 Veterinary Immunology and Immunopathology 163 3–4 pp 103-114

[31] Plan meropriiatii po profilaktike i borbe s leikoze krupnogo rogatogo skota na 2014-2019 gody v KFKh IP Zinchenko V.D.