Biotechnology methods for cattle leukemia elimination (experience of the Kemerovo region, Russia)

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Abstract. Cattle leukemia is a biological and socio-economic problem of Russian livestock production and is recognized as a potentially dangerous disease for humans. Currently, cattle leukemia is diagnosed in almost all countries of the world. Rate of infection in Russian herds varies from 10 to 70% in different regions and age categories. According to state veterinary report, leukemia was registered in 68 subjects of Russian Federation in 2016. Only Leningrad, Vologda, Arkhangelsk region and Stavropol region have recovered from leukemia. The paper outlines studies carried out in the farms of the Kemerovo region, in particular studies on the resistance of cattle to the leukemia virus, taking into account the age and physiological condition; impact of conditions, seasons on infection and incidence of cattle leukemia virus; features of the polymorphism of the BoLa-DRB3 gene in cattle herds of the Kemerovo region.

1. Introduction
Cattle leukemia (Bovine leukosis) is a chronic infectious disease of a tumorous nature, which occurs asymptomatically or is characterized by lymphocytosis and malignant growth of blood and lymphoid cells in various organs, caused by an RNA-containing virus [1, 2, 3, 4, 5, 6].

As part of the State Agricultural Development Program, Russian Ministry of Agriculture deploys a number of measures to diagnose leukemia, including purchase of disease diagnostics kits through the federal budget. Current Russian cattle population is 19.5 million. On average, about 60% of cows from all existing cattle are studied each year. Taking into account the average prices of laboratory tests for cow leukemia, the cost of researching this disease in our country alone is 14 billion rub. annually! [7, 8].

The economic damage caused by leukemia to livestock is significant due to a decrease in the quantity and quality of dairy and meat productivity, a decrease in breeding value of animals, losses as a result of culling of infected and sick animals, the disposal of carcasses, putting on the meat of young cows, the cost of carrying out recreational activities. Cows infected with leukemia milk production is reduced by 7-11%. According to a number of scientists, cows infected with leukemia virus constitute for a decrease of 20-35% of gross milk production; in terms of gross raw milk production 1.8 million tons are under-produced annually in Russia. It translates to a gross loss of 40 billion rub. The nutritional value of milk is also lost and that includes: general protein, most amino acids, fat content. Thus, based on the results of the scientists' research, it can be stated that leukemia exceeds such significant diseases as tuberculosis and brucellosis in economic damage [9, 10, 11, 12, 13].
Various immunological methods of diagnosis of CLV in the early stages of the disease, when there are no specific signs of the disease, have been developed and successfully used [14]. These methods include immunodiffusion (RID), neutralization (NR), complement binding reaction (RSC), immunoenzyme analysis (IA), polymerase chain reaction (PCR), syncytic inhibition tests, immunofluorescence and radioimmunological analysis [14, 15].

The goal of the research: to use biotechnology methods for leukemia elimination at livestock farms in the Kemerovo region.

Research tasks:
- to study the resistance of cattle to the leukemia virus, taking into account the age and physiological state of cattle in the Kemerovo region;
- to determine and assess the impact of conditions, seasons on infection and incidence of CLV (cattle leukemia virus);
- to identify the features of polymorphism of the BoLa-DRB3 gen in cattle herds of the Kemerovo region.

2. Research material and methods
The object of the study is cattle of black and mottled breed, owned by agricultural enterprises of the Kemerovo region.

During the work, 210 blood samples were examined, 70 samples were examined with immunodiffusion, and 70 samples were examined with PCR diagnostics.

Research on the DNA of the leukemia provirus of cattle (Bovine leucosis virus) (BLV) was conducted in the research laboratory "Biochemical, molecular-genetic research and breeding of farm animals" kits manufactured by VET FACTOR.

Genotyping of cattle by BoLA DRB3 loci was made in blood samples by polymerase chain reaction (PCR) and followed by analysis of polymorphism of the lengths of the fragments of restriction (PDRF) in the laboratory of My Gene LLC (Moscow).

The frequency of alleles was determined by taking into account the number of homozygotes and heterozygotes according to the corresponding allele according to the formula:

\[ P = \frac{2N_1+N_2}{2n} \]  

where: \( N_1 \) and \( N_2 \) - the number of homozygotes and heterozygotes for the allele studied, respectively;
\( n \) - sample size.

The statistical error for the allele frequency was determined by the formula:

\[ M_P = \sqrt{P_1 \cdot N} \]  

where: \( P_1 \) - allele frequency;
\( N \) - number of animals.

MS Excel (Microsoft office) was used for statistical data processing.

3. Results
The resistance of cattle to the leukemia virus, taking into account the age and physiological condition of cattle in the Kemerovo region, was studied according to veterinary reports.

The studies have shown that the percentage of positive animals detected by RID in the Kemerovo region varies in age and physiological condition. The percentage of RID positive cows ranged from 19.3 to 27.8, thus, the percentage of infected animals depends on age and physiological condition (table 1).
Table 1. Analysis of animal resistance to leukemia virus, taking into account age and physiological condition (in the Kemerovo region).

| Goals | Cows | Bred heifers | Heifers 6-16 months. |
|-------|------|--------------|----------------------|
|       | RID | % | Heads | RID | % | Heads | RID | % |
| Goals | Goals | Goals | Goals | Goals | Goals |
| 2016  | 40012 | 11154 | 27.8 | 9053 | 726 | 8.0 | 32785 | 3325 | 10.1 |
|       | 49590 | 13804 | 27.8 | 8880 | 770 | 8.6 | 36919 | 4632 | 12.5 |
| 2018 (1-2nd quarter) | 26805 | 5184 | 19.3 | 8178 | 1000 | 12.2 | 18721 | 2570 | 13.7 |

Data from autumn and spring studies on the example of the IE "Sinchenko V.D." of the MTF No. 1 and No. 2 are presented in tables 2, 3.

At two farms of entrepreneur "Sinchenko V.D." - MCF No. 1 (Sidorenkovo) and MCF No. 2 (Pomortsevo) - the following dynamics was observed: 4% in autumn and 8% in autumn.

No RID-positive cows have been identified in 2018. However, in the study of heifers and cows from 2016 to 2018, a higher percentage of RID-positive animals were registered in spring. This is possibly due to low immune resistance of the body.

Table 2. Dynamics of allocation of RID positive cattle MTF No. 1 (Sidorenkovo).

| Research period | Cow | Bred heifers | Heifers 6-16 months. |
|-----------------|-----|--------------|----------------------|
|                 | Heads | RID | Heads | RID | Heads | RID | Heads | RID | Heads | RID |
| 2016            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 397   | 38   | 9.6   | 115  | 18   | 15.7 | 296   | 28  | 9.5   |
| Autumn          | 396   | 22   | 5.6   | 92   | 6    | 6.5  | 326   | 35  | 10.7  |
| 2017            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 404   | 16   | 4.0   | 98   | 13   | 13.3 | 240   | 65  | 27.1  |
| Autumn          | 412   | 33   | 8.0   | 102  | 19   | 18.6 | 253   | 34  | 13.4  |
| 2018            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 393   | 0    | 0     | 68   | 5    | 7.4  | 270   | 64  | 23.7  |
| Autumn          | 413   | 0    | 0     | 91   | 0    | 0    | 254   | 32  | 12.6  |

Correlation between spring and autumn in the "Sinchenko V.D." of MTF No. 2 (Pomortsevo) is also not traceable (table.3). However, in the study of heifers and cows from 2016 to 2018, a higher percentage of RID positive animals were recorded in spring.

Table 3. Dynamics of allocation of RID positive cattle at "Sinchenko V.D." MTF No. 2 (Pomortsevo).

| Research period | Cow | Bred heifers | Heifers 6-16 months. |
|-----------------|-----|--------------|----------------------|
|                 | Heads | RID | Heads | RID | Heads | RID | Heads | RID | Heads | RID |
| 2016            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 401   | 10   | 2.5   | 70   | 7    | 10.0 | 250   | 20  | 8.0   |
| Autumn          | 404   | 7    | 1.7   | 60   | 0    | 0    | 286   | 2   | 0.7   |
| 2017            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 400   | 11   | 2.8   | 69   | 0    | 0    | 222   | 5   | 2.3   |
| Autumn          | 388   | 5    | 1.3   | 43   | 2    | 4.7  | 203   | 2   | 1.0   |
| 2018            |       |       |       |     |       |     |       |     |       |     |
| Spring          | 407   | 16   | 3.9   | 72   | 0    | 0    | 158   | 2   | 1.3   |
| Autumn          | 362   | 0    | 0     | 60   | 0    | 0    | 220   | 0   | 0     |
Interesting data was obtained from the analysis of impact of conditions on infection and morbidity of CLV (cattle leukemia virus) at the IE "Sinchenko V.D." MFF No. 1 (Sidorenkovo) (table 4). In 2016 and 2017, infection among tethered cows ranged from 11.9 to 15.2%. All RID positive cows and heifers were kept loose.

**Table 4. Effects of conditions on infection and incidence of CLV (cattle leukemia virus).**

| Research time | Cow | Bred heifers | Heifers 6-16 months. |
|---------------|-----|--------------|----------------------|
|               | Heads | RID | Heads | RID | Heads | RID |
|               | Heads | %   | Heads | %   | Heads | %   |
| 2016          |       |     |       |     |       |     |
| Tethered      | 396   | 60  | 15.2  | 0    | 0     | 0    |
| Loose         | 0     | 0   | 0     | 92   | 24    | 26.1 |
| 2017          |       |     |       |     |       |     |
| Tethered      | 412   | 49  | 11.9  | 0    | 0     | 0    |
| Loose         | 0     | 0   | 0     | 102  | 32    | 31.4 |
| 2018          |       |     |       |     |       |     |
| Tethered      | 413   | 0   | 0     | 0    | 0     | 0    |
| Loose         | 0     | 0   | 0     | 91   | 5     | 5.5  |

The same picture was observed in the "Sinchenko V.D." MTF No. 2 (Pomortsevo) (table 5).

**Table 5. Effect of conditions on infection and incidence of CLV (cattle leukemia virus).**

| Research time | Cow | Bred heifers | Heifers 6-16 months. |
|---------------|-----|--------------|----------------------|
|               | Heads | RID | Heads | RID | Heads | RID |
|               | Heads | %   | Heads | %   | Heads | %   |
| 2016          |       |     |       |     |       |     |
| Tethered      | 404   | 17  | 4.2   | 60   | 7     | 11.7 |
| Loose         | 0     | 0   | 0     | 0    | 0     | 0    |
| 2017          |       |     |       |     |       |     |
| Tethered      | 388   | 16  | 4.1   | 43   | 2     | 4.7  |
| Loose         | 0     | 0   | 0     | 0    | 0     | 0    |
| 2018          |       |     |       |     |       |     |
| Tethered      | 362   | 16  | 4.4   | 60   | 0     | 0    |
| Loose         | 0     | 0   | 0     | 0    | 0     | 0    |

Analysis of the data from the farms where research took place indicates that in the LLC "Mikhailovsky" at Prokopievsky district no RID positive animals have been identified since 2016. On the contrary, there is a tendency of increase of RID positive animals among tethered cows at "Sinchenko V.D." (MTF No. 1). But, unequivocally, we can't say that the percentage of RID positive cows depends on the way they are kept.

We examined serum samples for the presence of antibodies to the leukemia virus with immunodiffusion (RID) reaction in the district laboratories of the Kemerovo region.

The results of the research:
1. IE "Sinchenko V.D." - In a serological study of 35 blood serum samples in 35 samples, antibodies to the leukemia virus were detected.
2. LLC "Mikhailovsky" Ltd. - in a serological study of 35 samples of serum antibodies to the leukemia virus were not detected.

Whole blood samples were examined for the presence of DNA leukemia provirus with polymerase chain reaction (PCR).

The results of the research:
1. IE "Sinchenko V.D." - In 20 of the 35 whole blood samples, the DNA of the leukemia provirus was detected, which accounted for 57% of the samples studied, so in part of the animals, the DNA of the leukemia provirus was not detected.
2. In the study of animals belonging to St. Michael's LLC, no DNA samples of leukemia provirus were found in the samples of whole blood.

The problem of genetic resistance of cattle to disease is now very acute. Many scientists and teams are actively investigating the allele structure of the BoLA-DRB3 gene, which is responsible for resistance to infectious diseases, including the leukemia virus of cattle. [16, 17, 18, 19]

The BoLa-DRB3 Bos taurus gene has a high allele polymorphism. Currently 59 of its allele variants are discovered. Some are responsible for disease resistance, others - for susceptibility to this disease, the third have no associative links with the disease of animal leukemia (neutral alleles). Numerous studies have proven the link of alleles 0902, 2701, 2702, 2703, 2704, 2705, 2706, 0701 to resistance of animals to leukemia and the connection of alleles 1201, 1501, 1502, 1101, 0101, 0102 are linked with susceptibility to the disease. [20 for both].

A sample of 61 black and mottled cows bred in the Kemerovo region identified 25 allele variants. The presence of a large number of allele variants is a result of breeding and is consistent with the data of many authors [17, 21, 22, 23, 24, 25]. Of the alleles associated with leukemia resistance, three variants were identified - 0701, 0902, 2703. Of the alleles associated with leukemia susceptibility, there were 5 variants: 1001,1002,1101,1201 and 1501. Other alleles are considered to be neutral, thus no links with susceptibility or resistance to leukemia has not been proven.

The frequency of occurrence of different variants of alleles is presented in Table 6.

**Table 6. Frequency of different alleles of the BoLa-DRB3 gene in the studied number of cows.**

| Allele | Allele status | Number of carriers | Frequency of occurrence |
|--------|---------------|--------------------|------------------------|
| 0101   | Neutral       | 15                 | 0.139                  |
| 0501   | Neutral       | 1                  | 0.008                  |
| 0502   | Neutral       | 1                  | 0.008                  |
| 0601   | Neutral       | 2                  | 0.016                  |
| 0701   | Stable        | 1                  | 0.008                  |
| 0801   | Neutral       | 4                  | 0.041                  |
| 0902   | Stable        | 10                 | 0.090                  |
| 1001   | Sensitive     | 9                  | 0.074                  |
| 1002   | Sensitive     | 1                  | 0.008                  |
| 1101   | Sensitive     | 13                 | 0.123                  |
| 1103   | Neutral       | 1                  | 0.008                  |
| 1201   | Sensitive     | 16                 | 0.148                  |
| 1301   | Neutral       | 3                  | 0.025                  |
| 1401   | Neutral       | 1                  | 0.008                  |
| 14011  | Neutral       | 3                  | 0.025                  |
| 1501   | Sensitive     | 20                 | 0.172                  |
| 1505   | Neutral       | 1                  | 0.008                  |
| 1801   | Neutral       | 5                  | 0.041                  |
| 20012  | Neutral       | 1                  | 0.008                  |
| 2201   | Neutral       | 1                  | 0.008                  |
| 2703   | Stable        | 1                  | 0.008                  |
| 2801   | Neutral       | 1                  | 0.008                  |
| 4401   | Neutral       | 1                  | 0.008                  |
| 4701   | Neutral       | 1                  | 0.008                  |
| 5702   | Neutral       | 1                  | 0.008                  |

In the sample studied, there is a predominant distribution of alleles associated with susceptibility to leukemia and neutral to the disease.

Allele polymorphism of the BoLA-DRB3 gene in the study sample of healthy cows of SKP "Mikhailovsky" is represented by 17 alleles, animals with leukemia at "Sinchenko V.D." - by 18 alleles (table 7).
According to the total distribution of alleles of different status between samples of healthy and leukemia-sick animals, the difference is virtually not noted. That is, the population is saturated with different status alleles of the BoLA-DRB3 gene roughly in proportion. This suggests the need to develop a breeding program to saturate the genomes of the population of black and mottled cattle in the Kemerovo region with alleles associated with leukemia resistance. Currently, the selection of animals in farms is carried out only in order to increase the level of economic and useful features.

Table 7. Frequency of different alleles of the BoLa-DRB3 gene in healthy and leukemia cows.

| Allele | Allele status | Frequency of occurrence | Frequency of occurrence |
|--------|---------------|-------------------------|-------------------------|
|        |               | healthy animals          | leukemia-stricken animals|
| 0101   | Neutral       | 0.155q0.048             | 0.125q0.041             |
| 0501   | Neutral       | -                       | 0.016q0.015             |
| 0502   | Neutral       | 0.017q0.017             | -                       |
| 0601   | Neutral       | -                       | 0.031q0.021             |
| 0701   | Stable        | -                       | 0.016q0.015             |
| 0801   | Neutral       | 0.017q0.017             | 0.063q0.030             |
| 0902   | Stable        | 0.103q0.039             | 0.078q0.033             |
| 1001   | Sensitive     | 0.138q0.045             | 0.016q0.015             |
| 1002   | Sensitive     | 0.017q0.017             | -                       |
| 1101   | Sensitive     | 0.103q0.039             | 0.141q0.043             |
| 1103   | Neutral       | -                       | 0.016q0.015             |
| 1201   | Sensitive     | 0.103q0.039             | 0.188q0.048             |
| 1301   | Neutral       | 0.034q0.023             | 0.016q0.015             |
| 1401   | Neutral       | -                       | 0.016q0.015             |
| 14011  | Neutral       | 0.034q0.023             | 0.016q0.015             |
| 1501   | Sensitive     | 0.121                   | 0.219                   |
| 1505   | Neutral       | -                       | 0.016q0.015             |
| 1801   | Neutral       | 0.069q0.033             | 0.016q0.015             |
| 20012  | Neutral       | 0.017q0.017             | -                       |
| 2201   | Neutral       | -                       | 0.016q0.015             |
| 2703   | Stable        | -                       | 0.016q0.015             |
| 2801   | Neutral       | 0.017q0.017             | -                       |
| 4401   | Neutral       | 0.017q0.017             | -                       |
| 4701   | Neutral       | 0.017q0.017             | -                       |
| 5702   | Neutral       | 0.017q0.017             | -                       |

It is important to consider the genetic resistance of the population to leukemia at the level of distribution of genotypes frequencies. According to our data, the total frequency of resistance genotypes is 19.68%, and the genotypes of susceptibility - 65.57%, thus more than three times higher.

4. Conclusions
- Analysis of the resistance to leukemia virus in populations, taking into account the age and physiological condition, indicated that the percentage of RID positive cows most susceptible to cow leukemia, ranged from 19.3 to 27.8.
- In the case of untethered animals, the percentage of RID positive was within: cows 9.5 (2016) - 15.6% (2017); bred heifers 13.3 (2016) - 11.3% (2017); cows 11.8 (2016) - 15.8% (2017).
- In the case of untethered cows, the percentage of animals was the following: cows from 28.6 (2017) to 19.7% (2018 - for the half-year); bred heifers from 7.7 (2017) to 10.9% (2018); heifers 8.3-12.8%, respectively. Thus, the tendency of an increase in a number of RID positive animals can only be traced to tethered cows.
- Taking into account autumn and spring studies, it can be concluded that the percentage of RID positive cows was 24.1%. Correlation between spring and autumn is not traceable.
3. In the study of animals belonging to the IE "Sinchenko V.D.", DNA leukemia provirus was identified in 20 of 35 samples of whole blood, which constitutes 57% of the samples studied.

4. In the populations of black and mottled cows bred in the Kemerovo region, 25 allele variants have been identified. BoLA-DRB3-alleles and genotypes associated with leukemia susceptibility predominate. The cumulative frequency of neutral alleles in the sample studied was 37.5%, alleles associated with leukemia resistance - 10.6% and alleles associated with leukemia susceptibility - 52.5%. The total frequency of resistance genotypes is 19.68%, and the genotypes of susceptibility are 65.57%, thus more than three times higher.

Of all the alleles associated with leukemia resistance, only one variant was identified in a healthy herd - 0902 with a frequency of 10.3%. In a sample of leukemia infected cows, three variants of such alleles are identified - 0701, 0902, 2703 with a frequency of 1.6; 7.8 and 1.6%, respectively. The total frequency of alleles in the sample of sick animals was 11%. It should be noted that the frequency of allele 0902 in a sample of healthy animals is higher than in a sample of animals with leukemia - 10.3 against 7.8%.

The paper was prepared as part of the agreement with the Russian Ministry of Science No. 05.607.21.0208 "Developing genomic editing technology for the reproduction of high-value breeding dairy cattle resistant to leukemia virus" RFMEFI60718X0208.

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