8th International Scientific Conference
RURAL DEVELOPMENT 2017
Bioeconomy challenges
23–24 November, 2017, Aleksandras Stulginskis University

Proceedings of the 8th International Scientific Conference Rural Development 2017

Edited by prof. Asta Raupelienė

ISSN 1822-3230 / eISSN 2345-0916
eISBN 978-609-449-128-3

Article DOI: http://doi.org/10.15544/RD.2017.142

# SCREENING OF COMPLEX VERTEBRAL MALFORMATION (CVM) AND BOVINE LEUKOCYTE ADHESION DEFICIENCY (BLAD) IN THE AYRSHIRE CATTLE BREED IN THE NORTH CAUCASUS

**Vladimir TRUKHACHEV,** Department of Animal Nutrition and General Biology, Stavropol State Agrarian University, 355017, Zootechnical Lane, 12, Stavropol, Russian Federation, rector@stgau.ru
**Sergey OLEYNIK,** Department of Technologies of Animal production, Breeding and Selection, the Stavropol State Agrarian University, 355017, Zootechnical Lane, 12, Stavropol, Russian Federation, soliynik60@gmail.com *(corresponding author)*
**Nikolay ZLYDNEV,** Department of Animal Nutrition and General Biology, the Stavropol State Agrarian University, 355017, Zootechnical Lane, 12, Stavropol, Russian Federation, nz-kormlenec@yandex.ru
**Vitaliy MOROZOV,** Stavropol State Agrarian Universit, 355017, Zootechnical Lane, 12, Stavropol, Russian Federation, supermoroz@mail.ru

The Ayrshire dairy breed is renowned for producing large quantities of high quality milk and, therefore, is frequently used for crossbreeding. However, various hereditary anomalies caused by gene mutations have been recently recorded in calves produced by some Ayrshire sires. Most of these anomalies were shown to have a recessive inheritance pattern, thus imposing a threat of unpredictable dramatic changes in cattle genotypes under such factors as genetic drift, selection and inbreeding. The purpose of this study was to examine the susceptibility of the Ayrshire cattle bred in the North Caucasus to such hereditary abnormalities as complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD). The investigation was carried out on 16 cows with various phenotype and reproduction disorders that were selected based on a three-year veterinary observation of 440 livestock animals. The target group cows were generally the descendants of Hannulan Yaskiyri, Riihiviidan Urho Errant and O.R. Lihting. The results demonstrated that no animals under study were the carriers of these genetic disorders, which proved the mutant alleles of BLAD and CVM to be absent from the Ayrshire cattle livestock bred in the North Caucasus. Therefore, the sires of these cattle can be successfully used for breeding.

*Keywords: complex vertebral malformation (CVM); bovine leukocyte adhesion deficiency (BLAD); Ayrshire cattle breed*

## INTRODUCTION

Advances in reproductive biotechnology and the extensive use of artificial insemination have accelerated genetic progress in dairy cattle breeding, leading to increased milk production. Nevertheless, the selection emphasis has recently changed from increasing yield to enhancing quality of milk, which is known to be characterised by such indicators as protein and fat content. The Ayrshire breed can effectively produce large quantities of high quality milk and is, therefore, frequently chosen for crossbreeding. The average annual milk productivity of these cows reaches 5,500 kg, with the fat and protein content thereof exceeding 4 % and 3.2 %, respectively. Despite being less productive than the Holstein dairy breed, the Ayrshires demonstrate higher productive longevity of about 150,000 kg of milk. In Russia, the Ayrshire cattle population has been steadily increasing over the past 50 years. During this period, over 90,000 head have been imported from Scandinavian countries. At present, the Ayrshire breed accounts for 2.8% of the country's total cattle livestock (Annual report …, 2013p; Ayrshire Breed, FABA, 2017).

However, a major hurdle to the large-scale breeding of the Ayrshires has been the birth of calves with various phenotypic anomalies, which were shown to be hereditary and caused by gene mutations. To date, eighteen system and organ anomalies specific to the Ayrshire breed have been identified. Most of these follow the recessive inheritance pattern and represent a hidden genetic load, which impedes large-scale selection due to genetic drift, migration and inbreeding (Ernst, 2009).

Gene mutations, as a rule, involve those DNA segments that correspond to a particular gene. The underlying molecular mechanism is commonly associated with the deletion, addition or substitution of nucleotides. As a result, the expression of a mutant gene undergoes a change, thus disrupting various biochemical and physiological functions of the organism (Yearbook of breeding…, 2011).

The molecular basis of bovine leukocyte adhesion deficiency (BLAD) consists in a point mutation occurring in the coding portion of the CD18 gene and having the autosomal recessive mode of inheritance. A substitution of adenine for guanine in the DNA in the 383 position leads to amino acid substitution in the protein molecule. This results in impaired expression of the entire γ-integrin neutrophil surface protein chain, affecting the ability of leucocytes to perform the protective phagocytic function. Eventually, the animal develops an immunodeficiency condition causing it to become increasingly susceptible to infection. Innate immune deficiencies emerge due to the genetically determined inability of the animal to perform an immune response. Morphological and functional disorders of the cellular and humoral immune systems emerge in the organisms of these animals during the development of T and B lymphocyte populations, macro- and microphages, as well as during the formation of immunoglobulin's and the components of the complement system (Agerholm, 1995; Nasreen, 2009; Grobet, 1999; Nagahata, 2004; Oner et. al., 2004; Zhigachev, 2004).

In most European and American countries, special programmes are being implemented with the purpose of reducing the incidence of BLAD-syndrome alleles in cattle populations. Sires and herd replacements are regularly tested for mutant genes, with the results published in specialised catalogues (Turbina et. al., 2003).

A genetic test developed in 2002 and applied worldwide to detect latent CVM carriers has shown about 40% of sires in the Netherlands and France to be CVM carriers, with this proportion reaching 20% in the USA, 15% in Italy, 10% in Canada and 7% in Germany. Such widespread presence of mutant CVM alleles is certain to threaten the healthy genomes of other cattle populations.

In Russia, monitoring of this dangerous disease with the purpose of its eradication has been carried out since 2005 using an approach developed at the All-Russian State Research Institute of Animal Husbandry. During this period, breed bulls in Russia's stud farms have been tested for the incidence of CVM. It was shown that 3.7 % of these were CVM carriers. In other words, 1 out of 27 sires used for artificial insemination was a carrier of this latent defect (Kalashnikova et. al., 1999; Marzanova, 2011). The disease was brought to Russia largely due to the imports of elite heifers, sires or dairy semen from the Netherlands and the United States, and to a lesser extent from Germany and Canada. It should be noted that latent CVM carrier animals are still being imported to Russia (Citek et. al., 2006; Czarnik 2007, Ernst, 2009; Glazko, 1999; Zhigachev, 1987; 2004, Zakharov, 1995).

The aim of this study was to monitor the incidence of complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD) in the Ayrshire cattle livestock bred in the North Caucasus in order to effectively guide decision making in livestock development and breeding programmes.

## MATERIALS AND METHODS

The investigation involved 16 cows of the Ayrshire cattle bred in the North Caucasus that were selected from 440 cattle based on a veterinary observation. All the animals manifested various reproductive issues, such as abortions and excessive inseminations (12 cows), stillborn calves (3 cows) and deformity (1 cow). The target group accounted for 3.6% of the total cattle population and was represented mainly by the descendants of Ayrshire elite sires Hannulan Yaskiyri, Riihiviidan Urho Errant, O.R.Lihting (Fig. 1). The crossing was also achieved by the bulls: 34078 Yu.Erimies, 32772 I.Voypollo, 905 Urry, 36460 Mehtar, 26350 M.Inssi, 26705 Maytoralli, 32820 Yuh.Vikkeri, 39130 Vaaralan, 36455 Mainio, 36400 Kokkolan, 223 Hyalyu, 39984 L.Ipollo, K.Ayro 39213, 38459 Yu.Yusti, 38065 Gamlegard, 36455 Mainio, 1481 Luoko, 35144 R.Yunkari, 574 Iskamlake, 883730 Tukki, 905 Urry, 5167 Sunny, 7545057 Stealth, 10304106 LaBrie Wilton, 777850 Blekder Kilo, 901 Vertti, 36878 Ketolan, 782514 Kornelis, 34740 K.Iyvari, 38585 Ukaros.

The biomaterial for the DNA diagnostics comprised blood samples collected from the jugular veins of the animals under examination using VACUETTE vacuum systems. The blood samples were stored in a cold box at a temperature of above zero (2-4 °C) for no longer than 1–2 weeks.

The DNA diagnostics tests were carried out in a PCR cabinet equipped according to the state standard of the Russian Federation using the following PCR algorithm. In zone A, reception, registration and processing of the material were performed using a Hettich Universal 320R laboratory centrifuge, operating at a speed of 15.000 r/min within a temperature range of -20 °C – +40 °C. In zone B, DNA strands were isolated using a standard set of reactants (Isogen Lab Ltd., Moscow). In zone C, a reaction mixture was prepared for subsequent PCR-RV. In zone D, the PCR results were documented (Normative legal documents of PCR lab organization, 2017).

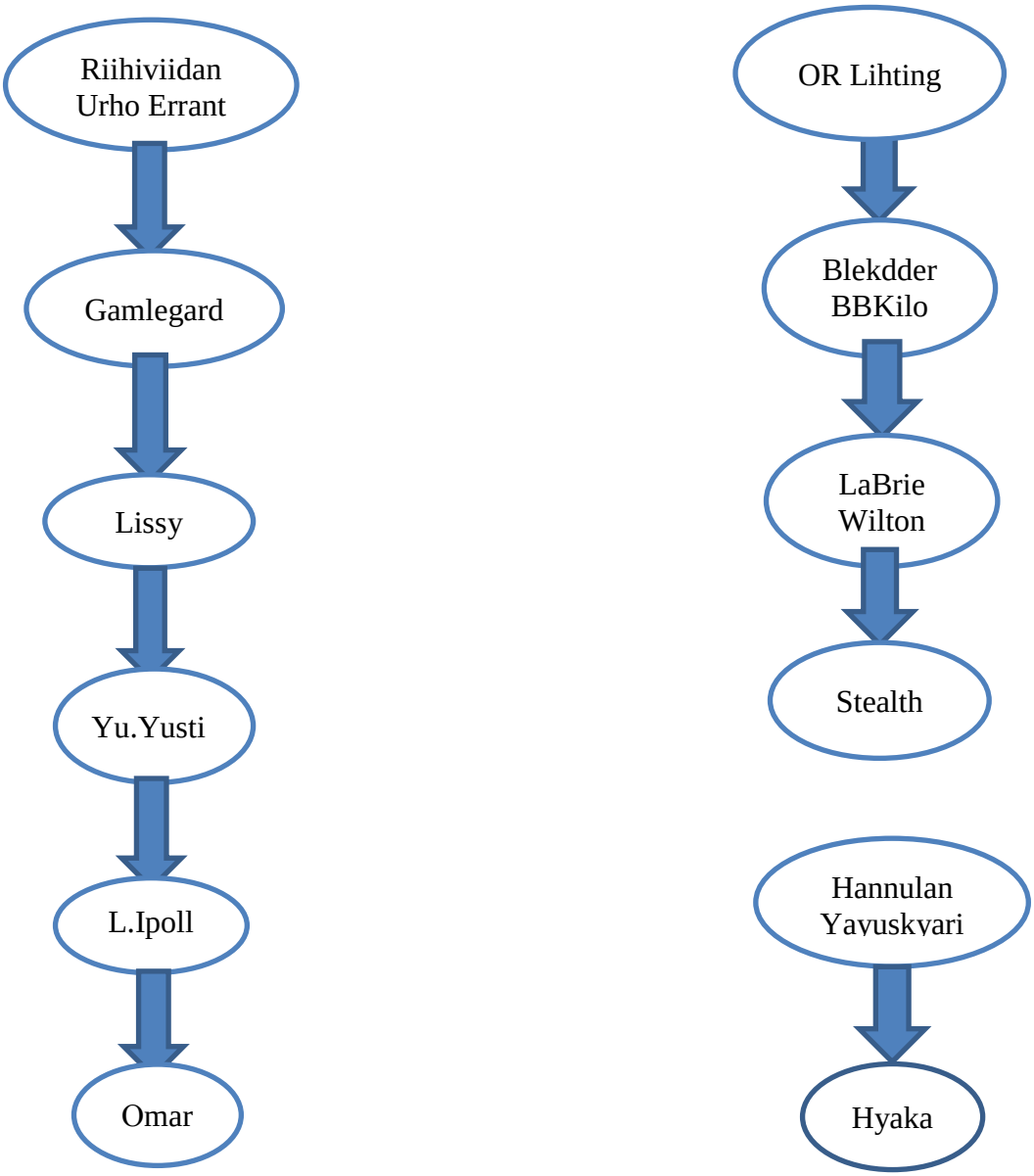


Figure 1. The genealogical scheme of the Ayrshire cattle bred in the North Caucasus

A real-time PCR was carried out on an ANK-32 (CJSC "Sintol", Moscow) using the thermal cycling programme shown in Table 1.

Table 1. Characteristics of thermal cycling parameters for the diagnostics of BL and BL CV mutant alleles causing leukocyte adhesion deficiency and complex spine abnormalities

| Steps | Temperature $^0$C | Time, sec | Detection | Repeats |
|---|---|---|---|---|
| Temperature maintenance | 94 | 180 | No | 1 |
| Cycling 1 | 94 | 20 | No | 10 |
| | 61 | 20 | No | |
| | 64 | 30 | No | |
| Cycling 2 | 94 | 20 | No | 30 |
| | 61 | 20 | No | |
| | 64 | 30 | By channels: Green, Yellow, Orange, Red | |

During the cycling process, denaturation, primer annealing and the synthesis of a new DNA strand occur. Under 10 repetitions, when cycling is 1, mutant alleles cannot be detected, since the data are not representative. However, under 30 repetitions (cycling is 2), the obtained data can be considered stable and comprehensive in terms of diagnostics.

The reaction mixture had the following composition: 50 mM KCl, 50 mM TRIS-HCl, 250 nM dNTP, 2.5 mM MgCl2 and 2.5 units of DNA polymerase Syntaq. The primer concentration was about 200 nM; for the fluorescent probes, a concentration of 100 nM was used. All the reagents were manufactured by CJSC "Syntol".

**RESULTS AND DISCUSSION**

In order to identify the mutant alleles of complex vertebral malformation (CVM) and bovine leukocyte adhesion deficiency (BLAD) in the Ayrshire cattle livestock bred in the North Caucasus by RT-PCR, we used the allele-specific TaqMan technology. Under this approach, an oligonucleotide complementary to the PCR product is used as a fluorescent probe.

According to the abovementioned technique, the probe is labelled with a fluorophore and a fluorescence quencher, with both terminal and internal oligonucleotide labelling being feasible. In the absence of a target, i.e. a sequence complementary to the probe, the fluorophore and the quencher are drawn close together, resulting in suppressed probe fluorescence. The quenching mechanism is known to be based on the fluorescence resonance energy transfer (FRET). In the presence of a DNA fragment complementary to the probe, the probe hybridises with an amplicon, leading to the cleavage of the latter by the 5'-exonuclease activity of the Taq DNA polymerase. The intensity of the signal in the fluorescence channel respective to the fluorophore increases proportionally to the amplicon accumulation with every PCR cycle.

In the present study, we used allele-specific probes for the detection of BL (BLAD) and CV (CVM) mutations in the genome fragment. For a particular mutation locus, a pair of probes was selected in such a way that one of them hybridised specifically at the circuit synthesis temperature with the normal allele (wild type), whereas the second probe was able to hybridise with the mutant allele. These probes were labelled with different fluorophores, with the reaction results being recorded in two different channels using a RT-PCR detection device.

For the detection of CT (normal) and CV (mutant) alleles, we used 2 channels, namely green and yellow. Normal CT alleles are known to produce signals along the green channel, which can be detected with the FAM fluorophore. On the other hand, mutant CV alleles are represented along the yellow channel and can be detected with the R6G fluorophore.

TL (normal) and BL (mutant) alleles were also detected using two channels: orange and red. The orange channel detects normal TL alleles with the FAM fluorophore, whereas the red channel traces mutant BL alleles with the Cy5 fluorophore (Table 2).

Table 2. PCR diagnostics of CVM and BLAD mutations: PCS is a positive control sample; NCS is a negative control sample; SS is a studied sample.

| Pos. | Description | Reactor | Type | CT FAM | CT R6G | CT ROX | CT Cy5 |
|---|---|---|---|---|---|---|---|
| A1 | PCS1 | CVM-BLAD | PCS | 28.95 | - | 25.67 | - |
| A2 | PCS2 | CVM-BLAD | PCS | 27.89 | 26.51 | 27.53 | 25.88 |
| A3 | PCS3 | CVM-BLAD | PCS | - | 27.26 | - | 26.46 |
| A4 | NCS1 | CVM-BLAD | NCS | - | - | - | - |
| A5 | 22154 | CVM-BLAD | SS | 27.51 | - | 25.52 | - |
| A6 | 21252 | CVM-BLAD | SS | 30.4 | - | 28.76 | - |
| A7 | 20371 | CVM-BLAD | SS | 29 | - | 29 | - |
| A8 | 22053 | CVM-BLAD | SS | 27.59 | - | 28.53 | - |
| B1 | 22160 | CVM-BLAD | SS | 28.75 | - | 28.51 | - |
| B2 | 22151 | CVM-BLAD | SS | 28.78 | - | 27.78 | - |
| B3 | 12180 | CVM-BLAD | SS | 28.06 | - | 28.24 | - |
| B4 | 21057 | CVM-BLAD | SS | 30 | - | 27.59 | - |
| B5 | 21218 | CVM-BLAD | SS | 29.89 | - | 25.7 | - |
| B6 | 29051 | CVM-BLAD | SS | 28.6 | - | 28.75 | - |
| B7 | 17196 | CVM-BLAD | SS | 28.87 | - | 28.78 | - |
| B8 | 27180 | CVM-BLAD | SS | 26.42 | - | 26.74 | - |
| C1 | 29293 | CVM-BLAD | SS | 32.3 | - | 27.64 | - |
| C2 | 27119 | CVM-BLAD | SS | 32.28 | - | 28.23 | - |
| C3 | 28238 | CVM-BLAD | SS | 29.77 | - | 28.72 | - |
| C4 | 10088 | CVM-BLAD | SS | 33.58 | - | 27.89 | - |

Figure 2 presents the PCR diagnostics results of BLAD and CVM genetic abnormalities in the studied cows of the North Caucasus Ayrshire cattle breed. The results obtained were interpreted on the basis of whether or not the fluorescence curve had crossed the respective threshold level.

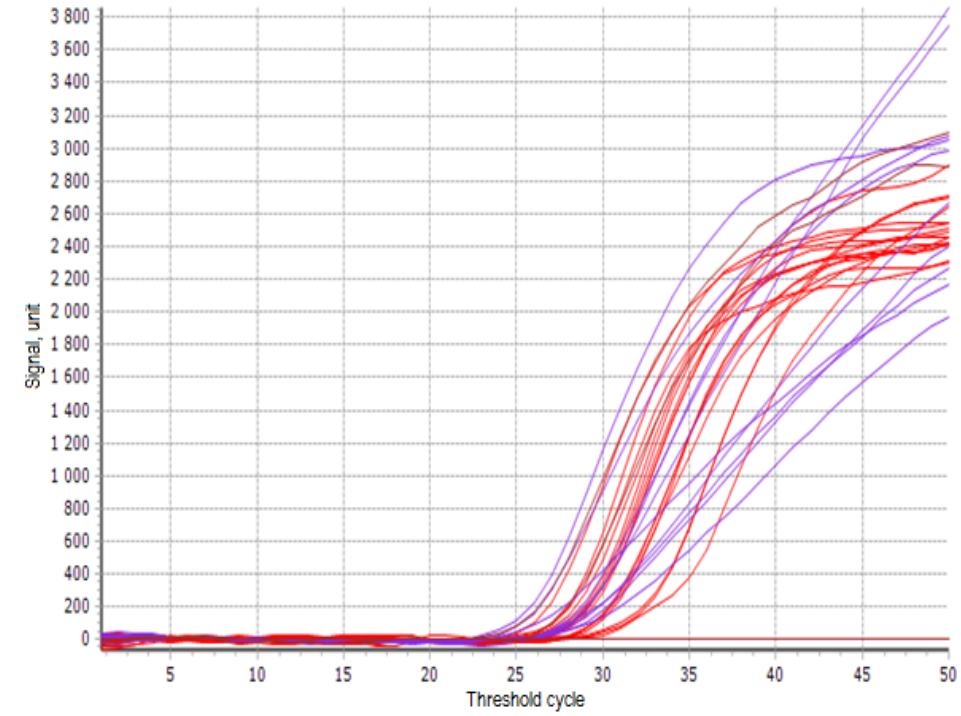


Figure 2. PCR diagnostics of BLAD and CVM genetic abnormalities in the studied cows.

The transition of the threshold is characteristic of the number of DNA copies in a single gene. When the number of DNA copies is optimal, the curve is located between two threshold values of 10 and 15 amplification cycles. Under a higher DNA concentration the curve moves to the left on the abscissa axis; conversely, the insufficient DNA concentration in the reaction shifts the curve to the right. All the samples, whose fluorescence graphs crossed the threshold line, were considered to be positive for a respective channel, i.e., containing the corresponding allele.

Table 3 displays the results of the PCR analysis for the detection of BLAD and CVM abnormalities in the studied cows. As is seen from the table, no mutant alleles were found.

Table 3. PCR analysis results of BLAD and CVM genetic abnormalities in the studied cows.

| Animal sequence number | **BLAD** | | **CVM** | |
|---|---|---|---|---|
| | TL (normal) | BL (mutant) | CT (normal) | CV (mutant) |
| 1 | + | - | + | - |
| 2 | + | - | + | - |
| 3 | + | - | + | - |
| 4 | + | - | + | - |
| 5 | + | - | + | - |
| 6 | + | - | + | - |
| 7 | + | - | + | - |
| 8 | + | - | + | - |
| 9 | + | - | + | - |
| 10 | + | - | + | - |
| 11 | + | - | + | - |
| 12 | + | - | + | - |
| 13 | + | - | + | - |
| 14 | + | - | + | - |
| 15 | + | - | + | - |
| 16 | + | - | + | - |

## CONCLUSIONS

**1.** The DNA diagnostics carried out on the studied animals of the Ayrshire cattle bred in the North Caucasus has detected no carriers of the following recessive mutations: bovine leukocyte adhesion deficiency (BLAD) occurring

in the CD-18 gene in locus 2244 and complex vertebral malformation (CVM) occurring in the 17th genetic linkage group in locus 2068.

**2.** It can, therefore, be suggested that the sires Hannulan Yaskiyri, Riihiviidan, Urho Errant and O.R.Lihting, used to breed the North Caucasus livestock, did not carry the abovementioned defective genes. The phenotype and reproduction disorders exhibited by the studied animals were likely to be of a non-hereditary nature.

**REFERENCES**


1. Agerholm, J.S., Houe, H., Jorgensen, C.B., Basse, A. 1993. Bovine leukocyte adhesion deficiency in Danish holstein-friesian cattle. II. Patho-anatomical description of affected calves. *Acta Veterinaria Scandinavica*, Vol. 34, pp. 237–243.
2. Annual report of the International Research Center IFCN (Dairy Research Center. Available at http://www.globalfarm.de/media/downloads/EXTRACT-Dairy-Report-2013.pdf
3. Arrayet, J.L., Oberbauer, A.M., Famula, T.R., Garnett, I., Oltjen, J.W., Imhoof, J., Kehrli, Jr., M.E., Graham, T.W. 2002. Growth of Holstein calves from birth to 90 days: The influence of dietary zinc and BLAD status. Vol. 80, Iss. 3, pp. 545–552.
4. Ayrshire Breed. The site of the Finnish Cattle Breeders Association FABA. Available at http://www.faba.fi/ru
5. Ayrshire Breed. The site of the Information and Trading system "Open pedigree market". Available at http://opr.lenagro.org/info/poroda/p2.php
4. Bukarov, N.G., Lebedev, E.U., Kaneev, A.Z., Morozov, I.M. 2004. Genetic monitoring - the methodology to improve the efficiency of cattle breeding. Materials of International Scientific Practical Conference: Past, present and future of animal production science, Vol. 1, Iss. 62, pp. 43–46. Dubrovitzy
6. Citek, J., Rehout, V., Hajkova, J., Pavkova, J. 2006. Monitoring of the genetic health of cattle in the Chech Republic. *Veterinarni Medicina*, Vol. 51, No. 6, pp. 333–339.
7. Czarnik, U., Grzybovski, G., Kaminski, S., Prusak, B, Zabolewicz, T. 2007. Effectiveness of a program aimed at the elimination of BLAD-carrier bulls from Polish Holstein-Friesian cattle. *Journal of Applied Genetics*, Vol. 48, pp. 375–77.
8. Ernst, L.K., Zhigachev A.I., Kudryavtsev, V.A. 2009. Monitoring of genetic burden in Black Spotted, Holstein and Ayrshire cattle breeds. Animal science, No. 3, pp. 5–10.
9. Glazko, V.I., Filenko A.P. 1999. The dynamics of the BLAD (immunodeficiency) spread in cattle. *Scientific-Theoretical Journal*. No. 2, pp. 41–43.
10. Grobet, L., Charlier, C., Hanset, R. 1993. Diagnostic genomigue de la BLAD (Bovine Leukocyte Adhesion Deficiency). Annales de Médecine Vétérinaire, Vol. 137, pp. 27–31.
11. Kalashnikova, L.A., Dunin, I.M., Glazko, V.I., Glazko, G.V., Ryzhova, N.V., Golubina, E.P.1999. DNA technology of farm animals' assessment. 148 p.
12. Marzanova, S.N., Alekseev, Ya.I., Konovalova, N.V., Turbina, I.S., Devrishov, D.A., Sochivko, D.G., Marzanov, N.S. 2011. Development of the diagnosis method of complex anomalies of the spine (CVM) by PCR in real time at the black-and-white cattle. *Scientific-Theoretical Journal: Biology Problems of Productive Animals*, No. 4, Spec. Iss, pp. 79–82.
13. Nagahata, H. 2004. Bovine leukocyte adhesion deficiency (BLAD): A Review. Journal of Veterinary Medical Science, Vol. 66, No. 12, pp. 1475–1482.
14. Nasreen, F., Altaf Malik, N., Naeem, Riaz M., Anver Qureshi, J. 2009. Detection and screening of bovine leukocyte adhesion deficiency in Pakistan using molecular methods. *Hereditas* Vol. 146, No. 2, pp. 74–78.
15. Normative legal documents of PCR lab organization. Available at. http://www.ld.ru/PCR/ilist-4339.html
16. Oner, Y., Keskin, A., Elmaci, C. 2010. Identification of BLAD, DUMPS, Citrullinamia and Factor XI Deficiency in Holstein Cattle in Turkey. *Asian Journal of Animal and Veterinary Advaces*, Vol. 5, No 1, pp. 60–65.
17. Turbina, I.S., Eskin, G.V., Turbina, G.S., Marzanov, N.S. 2003. The characteristic of bull- sires for gene BLAD-syndrome. Book of Abstracts of the 54th Annual Meeting of the European Association for Animal Production. Roma. Italy., pp. 60.
18. Vartapetyan, A.B. 1991. Polymerase chain reaction. *Molecular Biology*, Vol. 25, No. 4, pp. 926–936.
19. Yearbook of breeding work dairy at cattle farms in the Russian Federation. –Russian Research Institute of Animal Breeding, 2011. – 251p.
20. Zhigachev, A. I. 2004. Diseases of cattle in the XXI century – where are they from? *Our breeding work*, No. 3, pp. 9–11.
21. Zhigachev, A. I.1987. Problems of mutagenesis in modern agricultural animal populations. *Theses of the V conf. All-Union society of geneticists and breeders*, pp. 67–69.
22. Zakharov, I.A. 1995. Genetic maps of farm animals. Moscow. 35 p.