Chapter 26

# GENETIC ASPECT OF SOLVING ENVIRONMENTAL PROBLEMS OF ANIMAL HUSBANDRY BY REDUCING THE INCIDENCE OF NECROBACTERIOSIS IN CATTLE

Suprovych T. M., Karchevska T. M., Laiter-Moskaliuk S. V.

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## INTRODUCTION

According to the FAO (Food and Agriculture Organization of the United Nations), livestock is one of the largest sources of anthropogenic greenhouse gas emissions. Henning Steinfeld, a senior representative of the Food and Agriculture Organization of the United Nations (FAO), said: "Livestock production is one of the main contributors to the most serious environmental problems. Urgent action is needed to remedy the situation".

The livestock industry is responsible for 14.5 % of all greenhouse gas emissions – more than the entire transport sector. In addition, livestock production is responsible for 37 % of all methane emissions. According to Princeton University, methane has at least 30 times the greenhouse gas potential of carbon dioxide. Methane is released during the digestion process of ruminants. If we look at the data on food production in Europe, 83 % of all greenhouse gases produced by meat, dairy and egg production. What we eat directly affects our eco-footprint[1].

Very few studies have been conducted to determine the direct impact of animal diseases on greenhouse gas emissions. One of the few studies in this regard conducted in the United Kingdom. It assessed the impact of 15 endemic diseases and physiological disorders of dairy cattle (mastitis, lameness, infertility, etc.) on greenhouse gas emissions. The results showed that when animals are healthy, they produce an average of 3.8 % more milk (7831 compared to 7539 liters per year) and emit 1.6 % less $CO_2$[2]. Globally, livestock production is believed to have negative environmental impacts, affecting air, water, soil and ecology.

[1] How livestock farming harms the planet. What the livestock industry is silent about. URL: https://dnister.in. ua/articles/140717/yak-tvarinnictvo-shkodit-planeti-pro-scho-movchit-

[2] International Monetary Fund (2014). World Economic and Financial Surveys, 0256-6877. World economic outlook: recovery strengthens remains uneven. IMF, Washington, DC.

Lameness, along with mastitis and infertility, is the most common pathology in cattle. In dairy cattle, any abnormality causes an alteration in gait and is one of the main health and welfare issues on dairy farms[3]. It is generally accepted that it affects the performance of affected cows[4]. Reduced milk yields in lame cows are mainly due to reduced standing time during feeding and reluctance to move during feeding and milking[5]. This reduces the efficiency of feed use[6], which can worsen the environmental impact of the milk production process. Studies by Chen et al. found that lameness could increase the potential for global warming at the farm level, as well as the potential for acidification, eutrophication and fossil fuel depletion by 7–9 %[7].

The causes of lameness related to heredity, environment, feeding, cow behavior and other possible etiological factors[8]. For example, if cattle fed grain rather than grass, they emit less methane. However, a diet high in concentrates can also mean an increase in laminitis, which leads to painful lameness. High-yielding dairy cows are already predisposed to lameness due to selection breeding for high milk yields. Therefore, changes in the animals' diet or genome may well have negative consequences for welfare and health.

Most researchers consider necrobacteriosis (fusobacteriosis) to be the main factor among the causes of lameness in cattle. This is an infectious disease characterized by purulent necrotic tissue damage mainly in the lower limbs, especially in the corolla area. Thus, according to Peredera et al. 70 % of infectious hoof lesions are caused by necrobacteriosis[9]. Currently,

[3] Cha, E., Hertl, J. A., Bar, D., & Gröhn, Y. T. (2010). The cost of different types of lameness in dairy cows calculated by dynamic programming. *Preventive veterinary medicine*, 97 (1), 1–8. https://doi.org/10.1016/j.prevetmed. 2010.07.011

[4] Barnes, A. P., Rutherford, K. M., Langford, F. M., & Haskell, M. J. (2011). The effect of lameness prevalence on technical efficiency at the dairy farm level: an adjusted data envelopment analysis approach. *Journal of dairy science*, 94 (11), 5449–5457. https://doi.org/ 10.3168/jds.2011-4262

[5] Miguel-Pacheco, G., Kaler, J., Remnant, J., Cheyne, L., Abbott, C., French, A., Pridmore, T., & Huxley, J. (2014). Behavioural changes in dairy cows with lameness in an automatic milking system. *Applied Animal Behaviour Science*, 150, 1–8. https://doi.org/ 10.1016/j.applanim.2013.11.003

[6] Palmer, M., Law, R., & O'Connell, N. (2012). Relationships between lameness and feeding behaviour in cubiclehoused Holstein-Friesian dairy cows. Applied Animal Behaviour Science, 140, 3–4,121–127. https://doi.org/10.1016/j. applanim.2012.06.005

[7] Chen, W., White, E., & Holden, N. M. (2016). The effect of lameness on the environmental performance of milk production by rotational grazing. Journal of environmental management, 172, 143–150. https://doi.org/10.1016/j. jenvman.2016.02.030

[8] Anderson D. E. & Rogers G. M. (2001). Prevention of lameness in cow-calf operations. *Veterinary Clinics of North America: Food Animal Practice*, 17 (1), P.209–223. doi: 10.1016/s0749-0720 (15)30063-3

[9] Peredera, S. B., Kolotiy, M. V., Scherbakova, N. S., & Peredera, Zh. A. (2017). Monitoring of cattle necrobacteriosis in the agricultural company "Mayak" of Kotel'va district, Poltava region. *News of Poltava State Agrarian Academy*, 1–2, 126–128.

fusobacteriosis in animals is becoming widespread in countries with highly developed livestock production (USA, England, Germany) and in Ukraine. In recent years, the incidence of fusobacteriosis in cattle has become one of the most important infectious diseases in the structure of infectious pathology. In Ukraine, this disease has become widespread due to the importation of breeding animals from Western Europe, where necrobacteriosis has been recorded for a long time[10]. Necrobacteriosis accounts for 40–60 % of all diseases of livestock limbs[11]. Necrobacteriosis in cows on farms that do not use intensive livestock production technologies is not common and, as a rule, does not have the character of an enzootic. However, on farms that use intensive milk production technologies and year-round stall systems for keeping high-yielding cows, this infectious pathology is recorded much more often.

High-yielding Holstein-type cows now usually have to be culled at around 5 years of age due to lameness, mastitis or infertility related to their high productivity. Indeed, selective breeding for milk production has so skewed their metabolism that high yielding cows are "milked beyond endurance"[12]. Dairy cattle with a high genetic advantage for milk yield have an increased risk of lameness[13].

In this regard, it becomes apparent that there is a need to develop methodological approaches to assessing genetic susceptibility/resistance to necrobacteriosis as the main cause of lameness in cattle.

One of the possible ways to solve this problem is to develop modern molecular genetic express methods for studying the associations of BoLA-DRB3 gene alleles with various diseases, including necrobacteriosis.

The BoLA-DRB3 gene encodes class II antigens of the major histocompatibility complex of bovine tissue. Class II molecules are located on the surface of B cells, which, after intracellular processing, present foreign antigens to T cells to provide a humoral immune response. The allelic diversity of exon 2 of this gene is due to the need to bind a wide range of

[10] Rizhenko, V. P., Rizhenko, G. F., Gorbatyuk, O. I., et al. (2013). Infectious process and strategy of pathogenetic therapy in fuzobakteriozis. *Bulletin "Veterinary biotechnology"*, 23, 410–421.

[11] Demchuk, M. V., Knyshuk, P. V., Bojko, P. K., & Tkachuk, V. M. (2010). Prophylaxis characteristics of necrobacteriosis at livestock production farm. *Scientific Bulletin of the named after S. Gzhytskyi*, 2, 2 (1), 74–81.

[12] Sustainable food. Written evidence submitted by Compassion in World Farming. URL: https://publications. parliament.uk/pa/cm201011cmselect/cmenvaud/writev/food/sf36.htm

[13] Oikonomou, G., Cook, N. B., & Bicalho, R. C. (2013). Sire predicted transmissibility for conformation and yield traits and previous lactation incidence of foot lesions as risk factors for the incidence of foot lesions in Holstein cows. *Journal Dairy Science*, 96, 3713–3722. doi: 10.3168/jds.2012-6308

foreign antigens, which allows its alleles to be used as genetic markers for cattle[14].

The combination of molecular genetics and standard animal breeding techniques is essential in the selection and optimization of animal breeding programmers. Information on the genetic aspects of necrobacteriosis is rather limited. According to Boettcher et al., the estimated heritability of lameness does not exceed 10 %[15]. However, Oikonomou et al. indicates that for mastitis and laminitis, despite the low heritability of these diseases, selection based on genetic markers may be more important than conventional selection methods[16].

The aim of the study was to identify alleles of the BoLA-DRB3 gene, exon 2, associated with cow necrobacteriosis. The object of the study was the polymorphism of this gene in relation to resistance and susceptibility to necrobacteriosis. The subject of the study was the genetic structure of animals of the Ukrainian Black-and-White dairy cattle breed according to the gene polymorphism in connection with resistance and susceptibility to necrobacteriosis.

Obviously, the methods of selecting animals genetically resistant to necrobacteriosis will significantly reduce the risk of lameness, and healthy cattle herds will help reduce greenhouse gas emissions associated with the production of raw materials for the food industry and food itself.

## 1. BoLA-DRB3 gene: structure, function, polymorphism

The variability of MHC molecules realized at the population level. A single individual cannot have more than two varieties of products of each MHC gene, and therefore cannot fully recognize the full range of protein peptides. However, for the entire population, this possibility is possible due to the variability of the entire repertoire of MHC molecules[17].

[14] Singh, U., Deb, R., Alyethodi, R., et al. (2014). Molecular markers and their applications in cattle genetic research: A review. *Biomarkers and Genomic Medicine*, 6 (2), 49–58. https://doi.org/10.1016/j.bgm.2014.03.001

[15] Boettcher, P. J., Dekkers, J. C., Warnick, L. D., & Wells, S. J. (1998). Genetic analysis of clinical lameness in dairy cattle. *Journal of Dairy Science*, 81 (4), 1148–1156. doi: 10.3168/jds.S0022-0302 (98)75677-2

[16] Oikonomou, G., Michailidis, G., Kougioumtzis, A., Avdi, M., & Banos, G. (2011). Effect of polymorphisms at the STAT5A and FGF2 gene loci on reproduction, milk yield and lameness of Holstein cows. *Research in Veterinary Science*, 91 (2), 235–239. doi:10.1016/j.rvsc.2011.01.009

[17] Bennett R., Ijpelaar J. Economic Assessment of Livestock Diseases in Great Britain (2003). Final Report to Defra. The Department of Agricultural and Food Economics The University of Reading. URL: http://www.docstoc.com/docs/

Intensification of livestock production requires further development of the theoretical foundations and improvement of organizational forms of farm animal breeding by using new methods of assessing their genotypes. These methods include the use of various types of molecular markers. If a gene variant (allele) is found statistically significantly associated with a trait (disease, productivity, etc.), then it is possible to assert a genetically determined allele-trait association or the presence of its genetic marker. It is in this context that most researchers study the most polymorphic gene DRB3 of the MHC BoLA system.

Among the variety of genetic markers, this gene is unique, namely:

– Firstly, the relevance to the problems of disease resistance due to the high variability of the BoLA-DRB3 gene associated with the formation of the body's immune response to pathogens;

– Second, the significant polymorphism of the gene allows it to be used in the study of cattle biodiversity;

– Thirdly, due to its close localization to some productive loci, the search for associations of the gene with economically useful traits of cattle is ongoing.

To date, the allelic diversity of the BoLA-DRB3 gene has been studied for more than 40 *Bos Taurus* breeds, and research is ongoing.

The BoLA-DRB3 gene encodes class II antigens of the major histocompatibility complex of cattle. It is located on the outer side of the B-lymphocyte membrane in the region IIa of the DR sub-locus of the BoLA system and consists of six exons (Fig.1)[18]. Exon 2, which encodes the Bjorkman peptide-binding cleft, is the most polymorphic[19].To date, the structure and function of the BoLA-DRB3 gene have been studied in detail. The main efforts of researchers focused on identifying the peculiarities of the amino acid sequences of exon 2 in different cattle populations. Modern methods of sequencing various parts of the cattle genome make it possible to obtain accurate sequences and expand the allelic base for studying issues related to the MHC polymorphism.

[18] Russell, G. C., Smith, J. A. & Oliver, R. A. (2004). Structure of the BoLA-DRB3 gene and promoter. *European Journal of Immunogenetics*, 31, 145–151. https://doi.org/10.1111/j.1365-2370.2004.00461.x

[19] Gowane, G. R., Vandre, R. K., Nangre, M., & Sharma, A. K. (2013). Major histocompatibility complex (MHC) of bovines: an insight into infectious disease resistance. *Livestock research international*, 1 (2), 46–57.

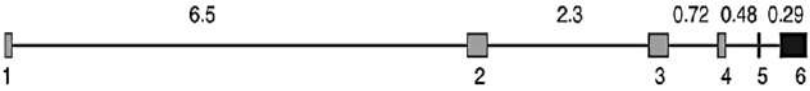


**Fig. 1. Scaled structure of the BOLA-DRB3 gene:**

1, 2 ... 6 are exons. The distances between exons in kbp indicated at the top. Exons shown as boxes, numbered below the gene, and introns as lines with sizes in kbp above.

MHC evolutionary processes have shaped a number of unique features of the BoLA-DRB3 gene. It characterized by a pronounced natural polymorphism. As of October 2023, the IPD-MHC website contains data on 390 allelic variants of the BoLADRB3 gene[20]. This is the highest polymorphism rate among all studied ruminant MHC loci.

The allelic spectrum of exon 2 of the BoLA-DRB3 gene (hereinafter referred to as BoLA-DRB3.2 alleles) has been studied for a significant number of cattle breeds, including four Ukrainian breeds[21]. The number of nomenclature (54 variants) of RFLP alleles in the studied breeds varies from 12 in the US Jersey to 36 in the Kalmykia. In most breeds, this figure varies between 20 and 28 variants. Among the identified alleles in different cattle populations, the most common is the BoLA-DRB3.2*24 allele.

To date, additional alleles have been identified that correspond to previously unknown combinations of DNA patterns. This expands the hypothetical base of RFLP alleles, which are classified as a group *without established nomenclature*[22]. For example, five alleles of this type were found in the Ukrainian grey breed: *jab, *jba, *jbb, *nad, *nda[23], six variants – for Ukrainian white-headed cattle: *nab, *mdb, *iab, *gbb, *fbd, *naa[24]. This is important genetic information, because one highly polymorphic gene is equivalent in terms of information content to a dozen two- or three-

[20] https://www.ebi.ac.uk/ipd/mhc/allele/list/?query=and(eq(organism.name,BoLA),eq(locus,%27DRB3%27))#panelAdvanced)

[21] Suprovych, T. M., Salyha, Yu.T., Suprovych, M. P., Fedorovych, E. I., Fedorovych, V. V. & Chornyj, I. O. (2022) Genetic Polymorphism of BoLA-DRB3.2 Locus in Ukrainian Cattle Breeds. *Cytology and genetics*, 6 (4), 319–330. doi:10.3103/S0095452722040089

[22] Oprzadek, J., Urtnowski, P., Sender, G., Pawlik, A., & Lukaszewicz, M. (2012). Frequency of BoLA-DRB3 alleles in Polish Holstein-Friesian cattle. *Animal Science Papers and Reports*, 30 (2), 91–101.

[23] Suprovych, T. M., Suprovych, M. P., Mokhnachova, N. B., Biriukova, O. D., Strojanovska, L. V., & Chepurna, V. A.

(2021). Genetic variability and biodiversity of Ukrainian Grey cattle by BoLA-DRB3 gene. *Regulatory Mechanisms in Biosystems*, 12 (1), 33–41. doi:10.15421/022106

[24] Suprovych, T., Suprovych, M., Biriukova, O., Chepurna, V., Karchevska, T., Kolodii, V., & Lesniak, Y. (2022). Genetic specificity of the white-headed Ukrainian breed according to the Bola-DRB3 gene. *Proceedings of the national academy of sciences of Belarus. Agrarian series*, 60 (1), 69–78. doi:10.29235/1817-7204-2022-60-2-69-78

characteristic genes. That is why using the results of the analysis of only one BoLA-DRB3 gene is sufficient to search for markers of disease/resistance and assess the level of biodiversity of breeds in general or individual herds or populations among themselves.

## 2. Identification of BoLA-DRB3.2 alleles associated with necrobacteriosis

### 2.1. Method for detecting the BOLA-DRB3 gene polymorphism

The method of restriction fragment length polymorphism (RFLP) was used to study the polymorphism of BoLA-DRB3.2 alleles. Its modern analogue PCR- RFLP refers to the enzymatic methods of analyzing SNPs (Single Nucleotide Polymorphism) based on the use of PCR. A group of researchers led by Van Eijk developed this method for cattle[25]. It consists in the fact that the study of genomic DNA is carried out by treating it with restriction enzymes, followed by electrophoretic separation of the resulting mixture and determination of the lengths of the resulting fragments. The endonucleases selected in such a way that the cleavage sites of the amplified DNA are strictly determined. Therefore, polymorphism at the DNA level leads to a different distribution of restriction sites along the respective DNA segments.

In the resulting restriction products, the number and length of homologous fragments will vary, and DNA polymorphism will be tested due to the presence of restriction sites of different lengths.

The PCR- RFLP method includes the following steps: isolation of genomic DNA, restriction of DNA with specific endonucleases, electrophoresis of DNA fragments, and identification of DNA patterns.

In the present study, a variant of two-step PCR using primers HLO-30, HLO-31, and HLO-32 used to detect the polymorphism of BoLA-DRB3.2 alleles. Restriction endonucleases *RsaI, HaeIII, and XhoII* used for restriction analysis of exon 2 of the BoLA-DRB3 gene. Based on the restriction patterns, 54 allelic variants of the BoLA-DRB3 gene were detected.

[25] Van Eijk, M. J. T., Stewart-Haynes, J. A., & Lewin, H. A. (1992). Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. Animal Genetics, 23 (6), 483–496. doi:10.1111/j.1365-2052.1992.tb00168.x

### 2.2. Methods of genetic and statistical analysis[26]

The allele frequencies were calculated taking into account the number of homozygotes and heterozygotes found for the corresponding allele according to the formula:

$$P(A) = 0.5\ (2N_{1i} + N_{2i})/n, \quad (1)$$

where $N_{1i}$ and $N_{2i}$ are, respectively, the number of homozygotes and heterozygotes for the studied (*i-th)* allele; *n is the* sample size.

An allele is a unit of genetic information that, under certain conditions, can serve as a DNA marker if a close link is established between it and a particular trait. In the case of a disease or resistance to a disease, there is an association or statistically significant relationship between them and BoLA alleles. To identify a DNA marker, it is necessary to establish the strength of the association and statistical significance between the frequency of gene carriers in groups of susceptible and resistant animals.

The strength of the association assessed by the relative risk (*RR*), which determines the probability of developing the disease in animals with the corresponding allele compared to those without it:

$$RR = \frac{f_b(1-f_k)}{f_k(1-f_b)} = \frac{ad}{bc} \quad (2)$$

where $f_b$ is the frequency of allele carriers among diseased animals;

$f_k$ – frequency of allele carriers in healthy animals;

*a* – diseased animals with the allele;

*b* – healthy animals carrying the allele;

*c* – sick animals with no allele;

*d* – healthy animals in which the allele is absent.

If $RR \geq 2$ indicates an association with susceptibility. At $RR \leq 0.5$, the presence of an allele indicates a close association with resistance. In this case, to highlight a positive association, the relative risk value is defined as *1/RR* with the opposite sign.

If the allele was not detected in the groups of susceptible or resistant animals, then one of the values of *a* or *b* is zero, and the value of the relative risk was determined by the Haldane formula[27]:

$$RR_c = (a+0.5)(d+0.5)/(b+0.5)/(c+0.5). \quad (3)$$

[26] Suprovych T. M., Suprovych M. P. Polymorphism of the BoLA-DRB3 gene as a marker of susceptibility to diseases of cattle: monograph. Kamianets-Podilskyi: PDATU. 2020. 185 c. http://188.190.33.55:7980/jspui/handle/123456789/7983

[27] Kleinbaum, D. G., Kupper, L. L., & Morgenstern, H. (1982). Epidemiological research. Lifetime Learning Publication. Belmont, Calif: Lifetime Learning Publications.

The correspondence criterion ($\chi^2$) indicates a statistically significant difference between the frequency of the allele in diseased and healthy animals:

$$\chi^2 = \frac{N(ad-bc)^2}{(a+b)(a+c)(b+d)(c+d)}. \quad (4)$$

For the two alternatives – *animals: sick ↔ healthy; allele: present ↔ absent* – with the number of degrees of freedom $dF = 1$, the marginal values of $\chi^2$ are: for $P \leq 0.05$

$\rightarrow \chi^2 = 3.84$; for $P \leq 0.01 \rightarrow \chi^2 \leq 6.63$; for $P \leq 0.001 \rightarrow \chi^2 = 10.8$.

If the detected alleles were no more than five, then the calculation of $\chi^2$ corrected by increasing their number by 0.5[28].

The value of $\chi^2$ makes sense when there are at least 20 animals in the sample and the conditions are met:

1. $(a+b)\times(a+c)/N>5$
2. $(a+b)\times(b+d)/N>5$
3. $(c+d)\times(a+c)/N>5$ (5)
4. $(c+d)\times(b+d)/N>5$.

The strength of associations for alleles that have minor deviations from the restrictions imposed on the established associations tested using two criteria:

1. The exact two-sided Fisher's exact test was used to assess the significance of differences in results depending on the impact of the risk factor;

2. Pearson's correlation coefficient used to assess the strength of the relationship between the risk factor and the outcome[29].

Statistical data processing carried out in the standard Microsoft Excel 2013 package using in-house programs and the integrated GenAlEx 6.503 add-on (http:// biology-assets.anu.edu.au/GenAlEx/Download. html).

### 2.3. Prevalence of necrobacteriosis among Ukrainian Black-and-White dairy cows and formation of experimental samples

The detection of BoLA-DRB3.2 alleles associated with necrobacteriosis was carried out for cows of the Ukrainian Black-and-White dairy breed on the basis of the breeding farm "Kozatska dolyna 2006» LLC Kamianets-Podilskyi district, Khmelnytskyi region. The farm received the status of a Ukrainian Black-and-White dairy breeding facility in 2007. Regular breeding activities allowed the farm to achieve a milk yield of over 7000 kg per cow.

[28] StatSoft® StatSoft® eTextbook. Basic statistics and tables: URL: http://statsoft.ru/ home/textbook/modules/ stbasic.html#spearson

[29] Medical statistics. URL: https://medstatistic.ru/calculators/calchi.html.

The study was conducted from 2015 to 2018. As a result, a research sample of 122 cows was formed. The group of resistant cows included 71, and 51 animals were diagnosed with necrobacteriosis. In 2020 and 2021, the diagnosis of cows that were in stage 1 or 2 of lactation at the time of the experimental sample formation was clarified.

As a result, data on the allelic spectrum of 4 cows were removed from the group of resistant animals, because at the time of culling, their diagnosis changed to the opposite. They were included in the group of cows with necrobacteriosis.

The main focus of the research farm's breeding work is to increase the proportion of heredity for the Holstein breed, for which the maternal herd is inseminated with semen from Holstein sires of the Elevation, Chief and Sovering lines (Table 1). Partial purchases of Holstein heifers are made.

Table 1

**Groups of cow origin by sire (bull) lines**

| № | Sire (bull) | | Inventory identification number | Number of daughters | Diagnosis | |
|---|---|---|---|---|---|---|
| | nickname (breed) | line | | | healthy | sick |
| 1 | Lemur (H) | Elevation | DK 1401362499 | 8 | 4 | 4 |
| 2 | Forge (H) | | CA 5440063 | 4 | 3 | 1 |
| 3 | Bob (H) | | US 133815562 | 8 | 2 | 6 |
| 4 | Andretti(H) | | US 136612814 | 7 | 5 | 2 |
| 5 | Buckshot (H) | | US 129444086 | 6 | 5 | 1 |
| 6 | Champ (H) | | US 134720997 | 10 | 3 | 7 |
| 7 | Vasari (H) | | FR 2931253623 | 13 | 8 | 7 |
| 8 | Vilmos (H) | | HU 3101733688 | 4 | 3 | 1 |
| 9 | Monarch (H) | | DE 1401837441 | 3 | 1 | 2 |
| 10 | Index (H) | | UA 8010911597 | 4 | 4 | – |
| | Total | | | 67 | 36 | 31 |
| 11 | Goldregen (H) | Chief | DE 350488769 | 4 | 2 | 2 |
| 12 | Fiasco (H) | | US 17089950 | 5 | 2 | 3 |
| 13 | Diamo (H) | | DE 1402173919 | 15 | 5 | 10 |
| | | Total | | 24 | 9 | 15 |
| 14 | Durant (H) | Sovering | US 2941264554 | 5 | 4 | 1 |
| 15 | Ronald (H) | | DE 5267723 | 1 | – | 1 |
| | | Total | | 6 | 4 | 2 |
| 17 | Maternus (H) | Valiant | DK 4195401081 | 8 | 4 | 4 |
| 18 | Snowy (CZ+G) | Fransa | UA 1741 | 4 | 4 | – |
| 19 | Barge (B&W) | | 161 | 3 | 2 | 1 |
| 20 | Caprice (B&W) | | 1189 | 2 | 2 | – |
| 21 | Locus (B&W) | | 6578 | 2 | 2 | – |
| 22 | Index (B&W) | | 1031 | 2 | 2 | – |
| | | Together | | 122 | 67 | 55 |

**Notes.** H – Holstein; B&W – Black and White

Cows with necrobacteriosis were detected by periodic examination of the herd. The main attention was paid to visual observation of animal behavior and limb condition. The diagnosis of necrobacteriosis was made on the basis of epizootic data, clinical picture of the disease and the results of bacteriological examination. Pathological material was sampled for isolation of *Fusobacterium necrophorum* in purulent necrotic lesions of the skin and adjacent connective and muscle tissues, mainly on the lower limbs.

In total, almost 2600 examinations were carried out and 397 cases of necrobacteriosis were detected, which was 15.3 %. The proportion of sick cows varied depending on the lactation number (Fig. 2).

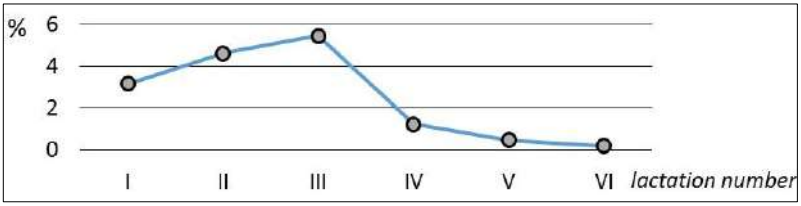


**Fig. 2. Dynamics of disease depending on lactation number**

Most often, necrobacteriosis occurred during the initial period of productive use, i.e., from the 1st to the 3rd lactation. At this stage, cows' immune system is becoming more intense and can be disturbed by sensitive factors (feeding, housing, care, etc.). At this stage, the genetic factor plays the greatest role in the animal's resistance to diseases in general and necrobacteriosis in particular. Therefore, the sample of cows susceptible to the disease included most young animals.

The decrease in sick cows of the fifth and sixth lactation is explained by the fact that the number of aged animals (7–9 years) did not exceed 4 % and, as a rule, only disease-resistant cows live to that age. Therefore, when forming the group of animals resistant to necrobacteriosis, preference was given to these cows.

### 2.4. Detection of DNA markers of resistance/susceptibility to necrobacillosis

The polymorphism of the experimental population of 122 cows was characterized by the presence of 31 (mean frequency 3.23 %) of 54 possible variants of the BoLADRB3.2 allele with different detection frequencies (Table 2). Six alleles were detected with a frequency of more than 5 %: *08, *10, *16, *22, *24, *28. Their total proportion was 49.6 %. The most common variant was *24 (18 %). Alleles *03, *07 and *23 had a frequency of 4.92 %.

This is a fairly high polymorphism rate, which requires attention to these alleles in the following analysis.

To identify candidate alleles for DNA markers in relation to resistance or susceptibility to bovine necrobiosis, it is necessary to compare allele frequencies in groups of healthy and sick cows. In this case, the reliability of the alternatives is assessed: the presence/absence of an allele in sick and healthy animals, as well as the multiplicity of the interaction between cause and effect sick/healthy, i.e. between the presence of an allele and the susceptibility of the animal to the disease. The data of the calculations for identifying significant associations are presented in Table 2.

Table 2

**Polymorphism of BoLA-DRB3.2 alleles and their associations with necrobacteriosis in Ukrainian Black-and-White dairy cows**

| BoLA-DRB3.2 alleles | Frequency, %. | | | $\chi^2$ | *RR* | Check for limited sample size by $\chi^2$ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | necrobacteriosis $n = 55$ | resistant $n = 67$ | of all $n = 122$ | | | $\frac{N}{(a+b)(a+c)}$ | $\frac{N}{(a+b)(b+d)}$ | $\frac{N}{(c+d)(a+c)}$ | $\frac{N}{(c+d)(b+d)}$ |
| *01 | 0,91 | 1,49 | 1,23 | 0.171 | 0.6 | 1.35 | 1.62 | 53.6 | 65.4 |
| *02 | 0,91 | 2,24 | 1,64 | 0.674 | $-2.53^{+}$ | 1.80 | 2.13 | 53.2 | 64.8 |
| *03 | 1,82 | 7,46 | 4,92 | $6.33^{*}$ | $-9.47^{+}$ | 4.96 | 5.23 | 50.0 | 61.0 |
| *04 | 0,91 | 2,24 | 1,64 | 0.674 | $-2.53^{+}$ | 1.80 | 2.13 | 53.2 | 64.8 |
| *07 | 6,36 | 3,73 | 4,92 | 0.437 | 1.52 | 4.96 | 6.13 | 50.0 | 61.0 |
| *08 | 4,55 | 6,72 | **5,74** | 0.561 | 0.644 | 6.31 | 7.23 | 48.7 | 59.3 |
| *10 | 3,64 | 6,72 | **5,33** | 1.2 | 0.505 | 5.86 | 6.61 | 49.1 | 59.9 |
| *11 | 1,82 | 1,49 | 1,64 | 0.04 | 1.23 | 1.80 | 2.20 | 53.2 | 64.8 |
| *12 | 1,82 | 2,99 | 2,46 | 1.33 | $-3.43^{+}$ | 2.25 | 2.62 | 52.7 | 64.3 |
| *13 | 1,82 | 4,48 | 3,28 | 1.4 | $-2.61^{+}$ | 3.61 | 4.13 | 51.4 | 62.6 |
| *15 | 0,91 | 2,24 | 1,64 | 0.674 | $-2.53^{+}$ | 1.80 | 2.13 | 53.2 | 64.8 |
| *16 | 10,9 | 1,49 | **5,74** | $10.5^{**}$ | $9.07^{-}$ | 6.31 | 8.84 | 48.7 | 59.3 |
| *18 | 2,73 | 0,75 | 1,64 | 1.5 | $3.81^{-}$ | 1.80 | 2.26 | 53.2 | 64.8 |
| *21 | 1,82 | 2,24 | 2,05 | 0.054 | 0.805 | 2.25 | 2.70 | 52.7 | 64.3 |
| *22 | 2,73 | 11,2 | **7,38** | $6.89^{**}$ | $-5.0^{+}$ | 8.11 | 8.11 | 46.9 | 57.1 |
| *23 | 8,18 | 2,24 | 4,92 | $4.81^{*}$ | $4.17^{-}$ | 5.41 | 7.18 | 49.6 | 60.4 |
| *24 | 20,0 | 16,4 | **18,0** | 0.167 | 1.17 | 18.93 | 22.38 | 36.1 | 43.9 |
| *26 | 1,82 | 2,99 | 2,46 | 0.352 | 0.594 | 2.70 | 3.20 | 52.3 | 63.7 |
| *28 | 8,18 | 6,72 | **7,38** | 0.013 | 0.94 | 7.21 | 8.52 | 47.8 | 58.2 |
| *32 | 1,82 | 2,24 | 2,05 | 0.054 | 0.805 | 2.25 | 2.70 | 52.7 | 64.3 |
| *36 | 0,91 | 4,48 | 2,87 | 2.85 | $-5.31^{+}$ | 3.16 | 3.56 | 51.8 | 63.2 |
| *37 | 3,64 | 2,99 | 3,28 | 0.084 | 1.24 | 3.61 | 4.39 | 51.4 | 62.6 |
| *48 | 1,82 | 1,49 | 1,64 | 0.171 | 0.602 | 1.35 | 1.62 | 53.6 | 65.4 |

**Note.** $^{*}P \le 0.05$; $^{**}P \le 0.01$; $^{+}$ resistance; – necrobacteriosis.

The table does not include alleles with a frequency of less than 1 %.

According to the relative risk size, 7 alleles *02, *03, *04, *12, *13, *15, *22 and *36 were identified with *RR* values ≤ -2, i.e., associated with resistance, and 3 alleles *16, *18 and *23 with *RR* values ≥ 2, i.e., associated with a predisposition to necrobacteriosis.

Consider the restrictions imposed on established associative links. Firstly, according to the $\chi^2$, only 4 alleles pass the test for the reliability of the established relationship, namely: *03 and *23 ($P < 0.05$), *16 and *22 ($P < 0.01$). Second, the alleles *8, *10, *16, *22, *23, *24 and *28 pass the test for sample size limitation according to formula 5.

An allele is considered to be associated with a particular trait if the following conditions are met: the frequency of detection in the sample is at least 5 %, the relative risk $RR \leq -2$ or $RR \geq 2$, the probable error according to the $\chi^2$ criterion $P < 0.05$ and the test for sample limitations is satisfied.

Only 2 variants meet these strict criteria. The BoLA-DRB3.2*16 allele indicates a close association with necrobacillosis, and the BoLA-DRB3.2*22 allele is associated with resistance to this disease.

As noted above, several alleles have minor deviations in terms of prevalence, reliability of established associations, or do not pass the test for sample limitations. Among them, two variants should be noted:

1. The BoLA-DRB3.2*03 allele is characterized by a slightly lower prevalence in the study sample $P(A) = 4.92$ % relative to the established limit of 5 % and slightly fails the test for sample limitation according to Clause 1 of Formula 5.
2. TheBoLA-DRB3.2 *23 allele also has a detection rate of $P(A) = 4.92$ %.

To address the question of the strength of associations, the possibility of using the *03 and *23 alleles and the possibility of using them as DNA markers was tested using the exact two-sided Fisher's test and the strength of association by Pearson's correlation coefficient (Table 3).

According to Fisher's exact criterion, both alleles satisfy the condition of reliability of the strength of association at the level of significance ($P < 0.05$). According to Pearson's coefficient, the BoLA-DRB3.2*03 allele shows an average strength of association, and the BoLA-DRB3.2*23 allele shows a weak one, which does not allow us to recommend both variants as DNA markers of susceptibility to necrobacteriosis.

Table 3

**Testing of BoLA-DRB3 alleles for association strength**

| BoLA-DRB3.2 alleles | *P(A), %* | $\chi^2$ | *RR* | $\underline{(a+b)(a+c)}\ N$ | Fisher's criterion | Pearson's coefficient |
|---|---|---|---|---|---|---|
| *03 | 4.92 | 6.33 | -9.47 | 4.96 | 0.012 | 0.222 |
| *23 | 4.92 | 6.03 | 4.86 | 5.41 | 0.035 | 0.195 |

To date, in addition to the presented study, only one work is known about the influence of the polymorphism of the BoLA-DRB3 gene on lameness in Chinese Holstein cows ($n$ = 435)[30]. The study was based on the identification of cow genotypes by restriction enzymes *BstUI*, *BstYI* and *HaeIII* (MBI fermentas, China). Based on the results of the study, the authors concluded that BoLA-DRB3.2 alleles could serve as candidates for lameness susceptibility in this population. The analysis of seven RFLP genotypes by *HaeIII* restriction enzyme showed statistically significant associations with laminitis in Chinese cattle.

## CONCLUSIONS

Thus, testing of multiplicative interaction in the groups "allele-disease" and "allele-resistance" with checks by $\chi^2$ tests, limited sample, Fisher's exact two-sided test and Pearson's correlation coefficient allowed to identify 2 alleles that have a significant relationship with susceptibility to necrobacteriosis in Ukrainian Black-and-White dairy cows. The BoLA-DRB3.2*16 allele can be recommended as a DNA marker associated with susceptibility to this disease ($P < 0.01$), and the BoLA-DRB3.2*22 allele can be recommended as a DNA marker associated with resistance ($P < 0.01$).

The identified markers allow us to change approaches to standard breeding methods. When developing breeding measures for the formation of genetically healthy herds, it is advisable to test sires (semen) and breeding cows for the BoLA-DRB3 gene for the presence of alleles associated with resistance/susceptibility to necrobiosis in their genotypes and to adjust the breeding process accordingly.

Genotyping for BoLA-DRB3.2 alleles in the initial phase of postnatal ontogeny provides a unique opportunity to form a disease-resistant herd. For this purpose, blood samples from heifers and bulls should be tested after birth to determine the allelic spectrum of BoLA-DRB3.2. If the *16 allele is detected in the genotype of cows, it is necessary to closely monitor the phenotypic manifestations of the disease and, if a negative diagnosis is confirmed, cull the cow from the dairy herd in advance. It is also desirable to carry out selection measures to accumulate the proportion of the BoLA-DRB3.2*22 allele in the maternal herd.

As already mentioned, any farm with a low lameness rate produces fewer greenhouse gas emissions, which has a positive impact on the climate situation. Conducting breeding activities based on the above conclusions will

[30] Sun, L., Song, Y., Riaz, H., & Yang, L. (2013). Effect of BoLA-DRB3 exon 2 polymorphisms on lameness of Chinese Holstein cows. *Molecular Biology Reports,* 40 (2), 1081–1086. doi:10.1007/s11033-012-2150-6

reduce the number of animals with necrobacillosis, which will have a positive impact on reducing lameness among cattle.


## SUMMARY

Lameness, along with mastitis and infertility, is the most common pathology among cattle, increasing the potential for global warming at the farm level, as well as the potential for acidification, eutrophication and fossil fuel depletion. Dairy cattle with a high genetic advantage in milk yield have an increased risk of lameness. In this regard, it becomes apparent that there is a need to develop methodological approaches to assessing genetic susceptibility/resistance to necrobacillosis as the main cause of lameness in cattle.

Molecular genetics methods are important in the selection and optimization of animal breeding programs. The aim of the study was to identify alleles of the BoLA-DRB3 gene exon 2 associated with necrobacteriosis in cows. To identify candidate alleles for DNA markers in relation to resistance or susceptibility to cattle necrobacteriosis, the polymorphism of BoLA-DRB3.2 alleles was studied using the PCR-RFLP method. Two alleles were found to be significantly associated with susceptibility to necrobacteriosis in Ukrainian Black-and-White dairy cows. The BoLA-DRB3.2*16 allele can be recommended as a DNA marker associated with susceptibility to this disease ($P < 0.01$), and the BoLA-DRB3.2*22 allele can be recommended as a DNA marker associated with resistance ($P < 0.01$).

Genotyping for BoLA-DRB3.2 alleles in the initial phase of postnatal ontogeny provides a unique opportunity to form a disease-resistant herd, which will lead to a reduction in greenhouse gas emissions, which will have a positive impact on the climate situation.


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Information about the authors:

**Suprovych Tetiana Mykhailivna,**
Doctor of Agricultural Sciences, Professor,
Head of the department of Animal Hygiene and Veterinary Support of the Cynological Service of the National Police of Ukraine,
Higher educational institution "Podillia State University",
12, Shevchenka str., Kamianets-Podilskyi,
Khmelnytskyi region, 32316, Ukraine

**Karchevska Tetiana Mykolaivna,**
Candidate of Veterinary Sciences,
Associate Professor at the Department of Infectious and Invasive Diseases,
Higher educational institution "Podillia State University",
12, Shevchenka str., Kamianets-Podilskyi,
Khmelnytskyi region, 32316, Ukraine

**Laiter-Moskaliuk Svitlana Vasylivna,**
Candidate of Veterinary Sciences,
Associate Professor at the Department the department of Animal Hygiene and Veterinary Support of the Cynological Service of the National Police of Ukraine,
Higher educational institution "Podillia State University",
12, Shevchenka str., Kamianets-Podilskyi,
Khmelnytskyi region, 32316, Ukraine