

T. Suprovich, T. Karchevska, R. Kolinchuk, V. Mizyk. Determination of alleles of BoLA-DRB3.2 gene associated with necrobacteriosis of the cows of Ukrainian Black-and-White Dairy cattle

The main objective of research "BoLA and disease" is the need to develop approaches and obtain reliable criteria which would allow to judge about animal genetic predisposition to the disease and about change of its immunological status in the development of pathological process. Genes of class II of main histocompatibility complex have the greatest association to diseases. Now 54 alleles of BoLA-DRB3.2 have been described by PCR-RFLP. The high level of allelic diversity of the gene is caused by necessity of tying a wide range of foreign antigens, which leads to the possibility of its use as a marker for various diseases of cattle.

This article presents the results of detecting alleles of BoLA-DRB3.2 gene, which have the expressed relationship with the disease of Ukrainian Black-and-White Dairy cows on necrobacteriosis and can be used as DNA markers of this disease.

Diagnosis of necrobacteriosis was set at the basis of clinical, pathological and epizootic data and laboratory results. The blood samples were taken from 114 cows, 43 of which had the disease. Spectrum of alleles of exon 2 of BoLA-DRB3 gene was studied by PCR. 54 alleles were determined in total. Alleles, which have a close relationship with susceptibility or resistance to necrobacteriosis and can be used as DNA markers, were established on indicators of frequency and relative risk (RR) with test on Pearson criterion (χ^2).

32 alleles were determined in the experimental group of animals. There were seven alleles with a frequency greater than 5%. The most often determined allele of BoLA-DRB3.2 was *24. It is present in 18% of the animals. And often determined alleles were *22 (7,9%) and *28 (7,5%). Limit higher than 5% was for alleles *08 and *09 (6,1%), *03 and *16 (5,3%). The lowest frequency of detection was for alleles *06, *25, *31 and *41 (0,4%).

Alleles of BoLA-DRB3.2 *24 (16,9%), *22 (10,6%), *28 (8,5%), *03 (7,7%), *08 and *10 (6,3%) were often determined in the group of healthy cows. Alleles *06, *14, *19, *25 and *51 weren't determined in this group. The animals with necrobacteriosis had often alleles *24 (19,8%), *16 (12,8%), *23 (8,1%), *8, *10 and *28 (5,8%). Alleles *01, *11, *21, *31 and *41 weren't in general.

In the three experimental groups 8 alleles were determined with a frequency of over 5% (all herd, healthy and diseased animals respectively). There are four alleles among them presented in all three samples: *08, *10, *24 and *28. Two "informative" alleles (*03 and *22) were found in every the 20th animal simultaneously in two groups of cows – healthy animals and in the total sample. Also two "informative" alleles *16 and *23 were simultaneously in the diseased cows and in the total sample.

11 alleles have significant association with susceptibility or resistance to necrobacteriosis on criterion of relative risk. There are 4 alleles *16 (24,1%), *18

(5,25%), *25 (5,04%) and *23 (4,41%), indicating the relationship with disease ($RR \geq 2$).

Four alleles of BoLA-DRB3.2 are significant on criterion χ^2 and have a sufficient test of validity for the studied biological objects. Allele *16 shows a very high level test of validity $P = 0,999$ ($\chi^2 = 16,6$). Three alleles *03 (4,93), *23 (4,86) and *22 (4,03) have a minimum acceptable test of validity for χ^2 for $P = 0,95$.

8 alleles: *3 (-7,7), *21 (-,44), *36 (-3,87), *22 (-3,57), *12 (-3,18), *1 and *11 (-3,13) and *26 (-2,51) indicate necrobacteriosis resistance ($RR \leq -2$).

Allele would be associated with the disease if the condition performed $RR \geq 2$ i $\chi^2 > 3,8$. There are two such alleles: *16 ($RR = 24,1$; $\chi^2 = 16,6$), *23 ($RR = 4,41$; $\chi^2 = 4,86$). Also "negative" alleles on risk of disease manifest are *18 (5,25) and *25 (5,08), but with insufficient validity of Pearson criterion (respectively 2,45 and 1,66).

Allele would be associated with the resistance to disease if the condition performed $RR \leq -2$ i $\chi^2 > 3,8$. There are 2 alleles associated with resistance to necrobacteriosis: *03 ($RR = -7,7$; $\chi^2 = 4,93$) and *22 ($RR = -3,57$; $\chi^2 = 4,03$). Also six alleles (*01, *11, *12, *21, *26 and *36) detected resistance to necrobacteriosis on high level of relative risk, but with insufficient validity.

It should be noted, that allele BoLA-DRB3.2*22, which proved to be a "positive" marker of resistance to necrobacteriosis, has a strong correlation with resistance to mastitis in cows of Ukrainian Black-and-White Dairy ($RR = -2,52$; $\chi^2 = 5,02$) and Ukrainian Red-and-White Dairy breeds ($RR = -4,66$; $\chi^2 = 11,11$) in previous studies.

The study of the distribution of alleles of exon 2 of BoLA-DRB3 gene at the Ukrainian Black-and-White Dairy cows, which were healthy and diseased by necrobacteriosis, revealed the alleles which had a close relationship with penchant to this disease (*16 and *23) and two alleles associated with resistance (*03 and *22). Given the fact that the research was conducted directly on animal blood DNA the detected alleles BoLA-DRB3 should be used as DNA markers in the analysis of susceptibility or resistance to necrobacteriosis of cows.

Keywords: cattle, necrobacteriosis, DNA markers, MHC, alleles