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COMPARISON OF *BOLA-DRB3* GENETIC DIVERSITY AMONG POLISH RED-AND-WHITE (PRW) AND POLISH HOLSTEIN-FRIESIAN (PHF) CATTLE*

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Abstract

Cattle breeding in Poland, including the maintenance of indigenous breeds, is exposed to the occurrence of many diseases of infectious and non-infectious etiologies. Bovine MHC (BoLA)-DRB3 is a highly polymorphic gene that plays an important role in the immune response to antigens. This pilot study aimed to analyze *BoLA-DRB3* genetic diversity among Polish Red-and-White (PRW) and Polish Holstein-Friesian (PHF) cattle. DNA sequencing revealed haplotypes for 48 out of 106 PRW and 10 out of 26 PHF cows. Analysis indicated 42 alleles detected in the PRW breed and 16 in the PHF animals. Interestingly, within the PRW breed three alleles BOLA03100|BoLA-DRB3*001:01|554, BOLA03113|BoLA-DRB3*009:02|270 and BOLA03302|BoLA-DRB3*049:01|236 were observed in 24 cows. Overall, the presented study demonstrated the genetic diversity of the *BoLA-DRB3* gene in the indigenous Polish Red-and-White cattle breed for the first time. The acquired results broaden the knowledge of *BoLA-DRB3* diversity in cattle, whose genetic variants promote resistance or susceptibility to viral or bacterial diseases.

Key words: cattle, indigenous breeds, MHC, sequencing, genetic variation

The high selection intensity of commercial dairy cattle breeds and an increase in crossbreeding with dual-type breeds has resulted in the displacement of indigenous breeds from breeding and production (Taberlet et al., 2011). This has contributed to a significant reduction in the genetic resources of native cattle breeds worldwide, including in Poland, which are less productive in milk than commercial breeds (Bett et al., 2013; Polak et al., 2021). As a consequence, resource conservation programs have been created and four Polish breeds, including Polish Red-and-White, which are a reservoir of biodiversity, are under their protection (http://www.bioroznorodnosc.izoo.krakow.pl/). The genetic potential of local Polish breeds relates to their better adaptation to the natural conditions of Poland, to longevity, better fertility and also resistance to diseases and parasites (Litwińczuk, 2011; Litwińczuk et al., 2015). The small number of Polish indigenous breed populations (PFHBiPM, 2020) necessitates particular care for optimal reproductive parameters, ensuring the possibility of reproducibility of the herd while limiting inbreeding. Among the factors limiting reproduction in native herds of cattle are viral, bacterial and parasitic diseases, some of which are closely related to reproduction.

The major histocompatibility complex (MHC) is involved in the immune response to infection and is a vital component of adaptive immunity. The products of class II MHC genes are molecules involved in the induction and regulation of the immune response which mainly play an essential role in antigen-presenting T-lymphocytes, also affecting the number and type of T-cells (Behl el al., 2012). In cattle, genes encoding proteins that form the MHC class II are known as BoLA (Bovine Leukocyte Antigen) and have been mapped to chromosome 23 (Takeshima and Aida, 2006). Class II of BoLA genes is divided into subregions: DRA, DRB, DQA and DQB. Within the DRB gene region, *DRBP1* is a pseudogene, while the functional expression of DRB2 has not been determined so far. An important role in response to antigens is played by the polymorphic *DRB3* gene, the prod-

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uct of which is a binding protein involved in the formation of antigen-antibody complexes. It is suggested that this gene plays a key role in the induction and regulation of the acquired immune response against viral and bacterial infections (Takeshima and Aida, 2006). In addition, a relationship between the frequency of selected DRB3 alleles and the resistance of cattle to infections like foot-and-mouth disease virus (FMDV) or bovine enzootic leukemia (BLV), or mastitis has been demonstrated (Sender et al., 2006; Hameed et al., 2008; Sharifzadeh and Doosti, 2012; Gowane et al., 2013; Pokorska et al., 2018). It has also been confirmed that the polymorphism of this gene is related to abortion in cattle (Schwab et al., 2009), a tendency to lameness (Sun et al., 2013), and possible immune response of animals to the vaccines used (Glass, 2004).

Recent research indicates that the genetic potential of indigenous cattle breeds consists of, among others, less intense one-way breeding selection and higher immune potential due to a better adaptation to local environmental conditions. However, infectious and invasive diseases in the Polish cattle population significantly reduce animal reproductive parameters, health and productivity. Therefore, the present project aimed to analyze haplotypes of the *BoLA-DRB3* gene and genetic diversity among Polish Red-and-White (PRW) and Polish Holstein-Friesian (PHF) cattle.

Material and methods

Animals, blood samples collection and DNA extraction

The experiment was conducted on Polish Red-and-White (PRW; n=106) and Polish Holstein-Friesian (PHF; n=26) cattle from three farms with milk performance recording systems. The Polish Red-and-White are under the Polish National Resource Conservation Program. Animals in each herd were kept in a free-stall barn and fed with a total mixed ration twice a day. Cows had free access to fresh water and salt licks. On the day of sampling, all animals were clinically healthy. Blood samples were collected from the jugular vein of each cow by the veterinarian supervising the herd. All experimental procedures were licensed by the 2nd Local Ethics Committee at Wrocław University of Environmental and Life Sciences, Poland.

The total genomic DNA was extracted from each blood sample using GenElute™ Blood Genomic DNA Kit (Sigma Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. After removing associated protein fragments, the purity and quantity of isolated DNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE).

DNA sequencing for *BoLA-DRB3*

Polymerase chain reaction (PCR) amplifications were carried out using a Bio-Rad C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). Each reaction (25) contained Maxima™ Hot Start Green PCR Master Mix (2X) (Thermo Fisher Scientific), primers and 50 to 100 ng of genomic DNA. Primers (forward 5'-cgctcctgtga(c/t)cagatctatcc-3' and reversed 5'-CAC CCC CGC GCT CAC C-3') were chosen from the literature (Miltiadou et al., 2003). The cycling conditions included an initial incubation of 3 min at 95°C followed by 34 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 1 min, and final elongation at 72°C for 5 min. All PCR products were purified using a Clean-Up Purification Kit (Applied Biosystems, Foster City, CA, USA).

The PCR products were sequenced in forward and reversed directions using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). All sequenced reactions were purified using a BigDye XTerminator Purification Kit (Applied Biosystems). Nucleotide sequences were determined using capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems).

Data analysis

The obtained sequences of the *BoLA-DRB3* gene were analyzed using BioEdit [http://www.mbio.ncsu.edu/BioEdit/bioedit.html]. The reference sequence of the *BoLA-DRB3* gene was taken from GenBank (JX2742321). Next, all the consensus sequences were aligned with ClustalW using the BioEdit Sequence Alignment Editor for the haplotyping analysis. Finally, the haplotyping was done using HaploFinder (http://www.bioinformatics.roslin.ed.ac.uk/haplofinder/) with all BoLA-DRB3 haplotypes (as for May 2022) from the Immune Polymorphism Database (IPD-MHC) (https://www.ebi.ac.uk/ipd/mhc/group/BoLA/) (Ellis et al., 2006).

Results

The BoLA-DRB3 sequence for all analyzed individuals was obtained during DNA sequencing (Supplementary Table 1 and 2). After sequencing, data was aligned and cut off to obtain a solid alignment block. Among all individuals, the analysis revealed haplotypes for 48 PRW and 10 PHF specimens, which is 45% and 38.5%, respectively (Supplementary Table 1 and 2). The level of heterozygosity in both breeds was 98% and 100% for PRW and PHF, respectively (Supplementary Table 1 and 2). Forty-two different *BoLA-DRB3* alleles were found within the PRW breed (Figure 1). Among the PRW cattle, the most frequent alleles were: BOLA03100|BoLA-DRB3*001:01|554, BOLA03113|BoLA-DRB3*009:02|270 and BOLA03302|BoLA-DRB3*049:01|236, each of which was found in 24 individuals (Table 1). In contrast, the least frequent alleles were: BOLA03106|BoLA-DRB3*005:01|260, BOLA03108|BoLA-DRB3*005:03|270, BOLA03124|BoLA-DRB3*016:01|801, BOLA03270|BoLA-DRB3*005:04|270, BOLA09909|BoLA-DRB3*100:01|269 and BOLA10166|BoLA-DRB3*005:08|269, which were found in only 1 individual. Among all anlyzed PHF individuals, 16 *BoLa-DRB3* alleles were

found (Figure 2). The most frequent allele in the PHF population was BOLA03116|BoLA-DRB3*011:01|270 (Table 1), while the least frequent alleles were: BOLA03110|BoLA-DRB3*007:01|270, BOLA03114|BoLA-DRB3*010:01|270, BOLA03115|BoLA-DRB3*010:02|271, BOLA03145|BoLA-DRB3*027:03|780, BOLA03286|BoLA-DRB3*027:07|249, BOLA09800|BoLA-DRB3*007:05|270, BOLA09804|BoLA-DRB3*027:18|268, BOLA09807|BoLA-DRB3*027:1268 and BOLA10036|BoLA-DRB3*014:03|269.

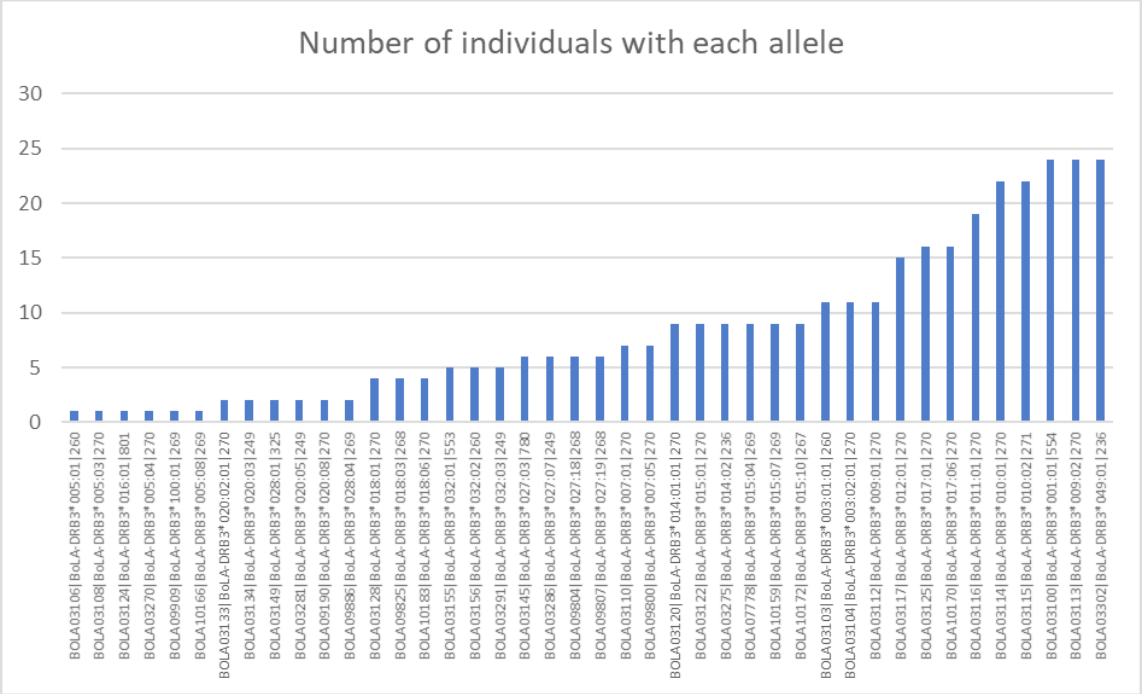


Figure 1. DRB3 alleles and its number found within Polish Red-and-White individuals

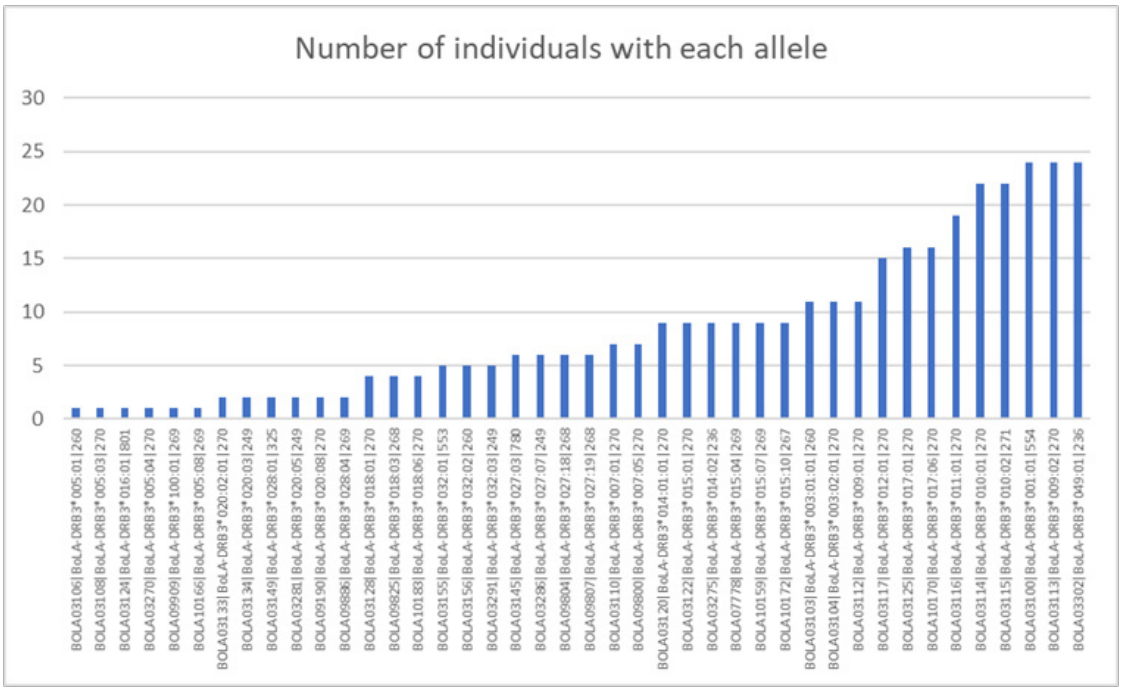


Figure 2. DRB3 allele and its number found within Polish Holstein-Friesian individuals

Table 1. Alleles with high frequency (>5%) found within Polish Holstein-Friesian (PHF) and Polish Red-and-White (PRW) population

DRB3 allele	PHF	PRW
BOLA03117|BoLA-DRB3*012:01|270	11.36%	4.26%
BOLA03100|BoLA-DRB3*001:01|554	13.64%	6.82%
BOLA03113|BoLA-DRB3*009:02|270	–	6.82%
BOLA03114|BoLA-DRB3*010:01|270	2.72%	6.25%
BOLA03115|BoLA-DRB3*010:02|271	2.72%	6.25%
BOLA03116|BoLA-DRB3*011:01|270	18.18%	5.40%
BOLA03120|BoLA-DRB3*014:01:01|270	13.64%	2.56%
BOLA03275|BoLA-DRB3*014:02|236	13.64%	2.56%
BOLA03302|BoLA-DRB3*049:01|236	–	6.82%

Discussion

Polish Red-and-White cattle have been bred in Poland for 100 years and constitute approx. 6% of the cattle population in Poland. These animals are characterized by resistance to diseases, good health, longevity and good adaptation to harsh environmental conditions (Majewska, 2006). In the presented pilot study, a comparison of *BoLA-DRB3* genetic diversity among Polish Red-and-White (PRW) indigenous cattle and commercial Polish Holstein-Friesian cattle was carried out. To our knowledge, this is the first study that examined *BoLA-DRB3* allelic variants in PRW cattle. Numerous studies have analyzed *BoLA-DRB3* genetic diversity in many different breeds, including native cattle (Takeshima et al., 2003; Oprządek et al., 2012; Pokorska et al., 2018; Takeshima et al., 2018; Bohórquez et al., 2020). In our study, the most frequent *DRB3* allele in PHF cattle (BOLA03116|BoLA-DRB3*011:01|270) was more than three times less frequent in the PRW population. The same difference in BOLA03116|BoLA-DRB3*011:01|270 allele frequency between HF and local breeds was found by Takeshima et al. (2018). In addition, Takeshima et al. (2015) demonstrated that other HF populations showed a high frequency of BOLA03100|BoLA-DRB3*001:01|554 and BOLA03116|BoLA-DRB3*011:01|270 *DRB3* alleles compared to local breeds (Takeshima et al., 2015) however, this breed is susceptible to tropical endemic pathogens and suffers from heat stress and thus fewer Holstein populations are raised in tropical areas. The bovine major histocompatibility complex (BoLA. In the present study, another *DRB3* allele (BOLA03113|BoLA-DRB3*009:02|270) had high frequency in the analyzed PRW cattle. Similar research concerning Bolivian HF and Yacumeño cattle populations showed a higher frequency of this allele in both breeds, Bolivian HF and Yacumeño cattle populations analyzed by Takeshima et al. (2015). These authors suggested that the high frequency of BOLA03113|BoLA-DRB3*009:02|270 allele and low frequency of BOLA03100|BoLA-DRB3*001:01|554 allele may be the result of either adaptation to tropical conditions or intensive use of only a few bulls (carriers of those alleles). In light of the results presented in this paper, the latter hypothesis may be the correct one. Furthermore, as shown by Yoshida et al. (2009), alleles BOLA03116|BoLA-DRB3*011:01|270 and BOLA03117|BoLA-DRB3*012:01|270 were associated with resistance to mastitis, while alleles BOLA03100|BoLA-DRB3*001:01|554 and BOLA03122|BoLA-DRB3*015:01|270 revealed an association with an increased incidence of mammary gland inflammation. Alleles BOLA03116|BoLA-DRB3*011:01|270 and BOLA03117|BoLA-DRB3*012:01|270 showed high frequency in both analyzed cattle populations in this study. However, the substantial difference in BOLA03100|BoLA-DRB3*001:01|554 allele frequency should be noted, as in the PRW cattle population its frequency was three times lower than in PHF cattle. Allele BOLA03122|BoLA-DRB3*015:01|270 was not found within PHF cattle, while its frequency in the PRW population was relatively low, amounting to 2.56%.

In summary, a complete profound understanding of gene variants present in indigenous cattle breeds, which are usually more resistant to different diseases, could be used in the genetic improvement of other breeds. The availability of these data could contribute to refining selective breeding programs. Further investigation into the association between the *BoLA-DRB3* gene and particular resistance or susceptibility to viral or bacterial diseases in Polish Red-and-White cattle should be continued.

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