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DRB3 Gene Polymorphism and Somatic Cell Count in Milk of Jersey Cows

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Abstract: The aim of this study was to investigate associations between BoLA-*DRB3* polymorphism and Somatic Cell Count (SCC) in milk, daily milk yield and milk fat and protein percentage content. DNA used in the study was obtained from 184 Jersey cows from Wielkopolska region in Poland. The frequencies of *DRB3*/*Hae*III alleles and genotypes were determined. Four alleles of the following frequencies were found: A-0.560; B-0.195; D-0.008 and E-0.236. The association between DRB3/*Hae*III polymorphism and SCC (transformed into a logarithmic scale) was analysed with regard to parity, season, stage of lactation, year of study and cow as sources of variability. Statistically significant associations were found with regard to all the analysed factors. The highest SCC was recorded in milk of EE-genotype cows, whereas the lowest SCC was found in BB-genotype cows. The study also included the effect of *DRB3*/*Hae*III genotype on daily milk yield and fat and protein content in milk. *DRB3*/*Hae*III genotype was found to be non-significant for these traits. The results obtained in this study suggest that BoLA-*DRB3* is a candidate marker for SCC and, consequently, for mastitis re sistance/susceptibility in dairy cows.

Key words: *DRB3* polymorphism, somatic cell count, milk utility traits, jersey, cow

INTRODUCTION

Mastitis (udder infection) poses a major problem in the management of dairy cows. Producers suffer huge losses due to veterinary treatment costs or, in some cases, necessary culling of infected animals. Milk of cows afflicted with mastitis is not suitable for consumption, which also leads to a reduction in the profitability of the production process.

The occurrence of *mastitis* is influenced by both environmental and genetic factors as well as interactions between them (Lindhe and Philipsson, 1998). Susceptibility to *mastitis* is also associated with the anatomical structure of the udder (including the structure of the teats), cow hygiene and milking technology (Smith and Hogan, 1993; Faye *et al*., 1994; Elbers *et al*., 1998; Barkema *et al*., 1999; Whitaker *et al*., 2000; Persson *et al*., 2003; Koster *et al*., 2006a, b).

Mastitis prevention in dairy cows by improving environmental conditions has proved to be ineffective. Despite the considerable advances in cow-keeping technologies, the incidence of udder infection remains high, in particular in high-production herds (Oltenacu and Ekesbo, 1994). Therefore, more effective methods of preventing this disease have to be found. One of them is to base cow breeding on the differences in hereditary susceptibility to *mastitis*. The differences are observed among breeds as well as individual animals. Thus, it seems reasonable to use Marker Assisted Selection (MAS).

Several studies have been conducted with the aim of locating genes that affect resistance to *mastitis* in cattle. Immune response to this disease is induced and regulated by, among other factors, Major Histocompatibility Complex (MHC) gene products.

In cattle, MHC (BoLA) genes (which are located on chromosome 23) have been extensively evaluated as candidate markers for associations with various bovine diseases and immunological traits (Andersson and Davies, 1994; Lewin, 1996; Lewin *et al*., 1999). Such associations have been reported by many authors (Glass *et al*., 1991; Xu *et al*., 1993; Dietz *et al*., 1997 a, b; Sharif *et al*., 1998; Maillard *et al*., 2003). Specifically, associations between both class I and II genes and the incidence of mastitis have been found (Oddgeirsson *et al*., 1988; Lunden *et al*., 1990; Schukken *et al*., 1994; Sharif *et al*., 1998).

Moreover, bovine MHC also appears to influence other than immunological traits, namely milk yield growth and reproduction. Sharif *et al*. (1999), Machado *et al*.

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(2005) and Vukasinovic *et al.* (1997) found statistically significant associations between DRB3 alleles and milk utility traits.

In cattle, the MHC gene is the most widely studied class II gene as it is extremely polymorphic with over 90 different alleles detected (Takeshima *et al.*, 2001). In recent years, the studies on *DRB3* polymorphism have benefited from the development of PCR-RFLP methods (Van Eijk *et al.*, 1992; Gelhaus *et al.*, 1995). *DRB3* polymorphism is recognized by three restriction enzymes (*Rsa*I, *Bst*YI and *Hae*III) and is characterized by a large number of gene variants.

The aim of this study, was to search for associations between *DRB3* gene polymorphism and susceptibility/ resistance to mastitis and milk production traits in a population of Jersey cows. The primary trait used to evaluate susceptibility to mastitis is Somatic Cell Count (SCC). SCC enables indirect selection for resistance to mastitis. A number of factors justify the use of SCC: SCC is relatively easy and inexpensive to collect; estimates of genetic correlations between SCC and clinical mastitis are sufficiently high; the heritability of SCC is 2 to 3 times greater than the heritability of clinical mastitis; SCC is an indicator of not only clinical but also subclinical infections.

MATERIALS AND METHODS

The study included a herd of 184 dairy Jersey cows from Wielkopolska region in Poland. All the animals were kept in identical environmental conditions. They were fed standard feed ratios and seasonally (in spring and summer) were put out to pasture. The cows were milked twice a day with the use of a pipeline milking machine. The herd's milk yield was evaluated with the A4 method in compliance with the recommendations of the International Committee for Animal Recording (ICAR). The data concerning SCC in milk, fat and protein content and daily milk yield were collected in the years 1998-2002 on the basis of monthly milking tests, representatively sampled (2513 samples) from both of two milkings performed at the same day time for each cow. SCC and fat and protein content in the samples were determined with an instrumental method in accordance with the PN-EN ISO/IEC 17025 standard, using Combifoss equipment (including Fossmatic 5000 and MilkoScan 6000 apparatuses, Foss, Hillerod, Denmark). The analyses were carried out in the ICAR and COFRAC certified laboratory of milk analyses in Krotoszyn, Poland. Percentage fat content was determined per weight ratio. Protein and fat content was expressed in g 100 mL^{-1} (%) and somatic cells in PCs mL^{-1}. Calibration was completed on the basis of standardized test samples from the Central Laboratory of the National Animal Breeding Centre in Parzniew, Poland (ICAR and COFRAG certificates, CECALAIT member). The Gerber and Röse-Gottlieb method was used as a reference method for determining fat content and the Kjeldahl method was used for determining protein content.

Analytical method: Peripheral blood was collected into tubes coated with an anti-coagulant and afterwards DNA was isolated. The isolation of DNA from the whole blood was done with a Master Pure™ DNA Purification Kit for Blood (Epicentre, USA).

The isolated DNA was used for a two-stage PCR amplification of the DRB3 gene fragment of 284 bp (base pair) with the use of the following primers proposed by van Eijk *et al.* (1992) (http://www.projects.roslin.ac.uk/bola/drb3pcr.html#table):

First stage primers:

Forward: 5'-ATCCTCTCTCTGCAGCACATTTCC-3'
Contains 7 nucleotides of the 5' and 3' ends of exon 2 plus intron sequences.
Reverse: 5'-TTTAAATTCGCGCTCACCTCGCCGCT-3'
Contains 8 nucleotides of the 5' and 3' ends of exon 2 plus intron sequences.

Second stage primers:

Forward: 5'- the same as in the first stage
Reverse: 5'-TCGCCGCTGCACAGTGAAACTCTC-3
Consists entirely of nucleotides of the 3' end of exon 2 and has an 8 basepair overlap with 3' end of first stage reverse primer.

The PCR reaction was carried out in Biometra termocyclers (Whatman Biometra GmbH, Gottingen, Germany) according to van Eijk *et al.* (1992) using Fermentas reagents (Fermentas, Madison, USA) and Proligo primers (Proligo France SAS). Restriction analysis of the amplified fragment was performed with the RFLP method using *Hae*III enzyme (for 3 h, with 5 units 20 μL^{-1}, at 35°C). The restriction fragments were separated on high-resolution 4% agarose gels (MetaPhor®, Cambrex Bioscience, Rockland, Inc., USA) containing ethidium bromide. Then, the fragments were visualized under UV light and their length was determined using a gel imaging and documentation system (Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany).

Statistical methods: The frequencies of *DRB3*/*Hae*III alleles and genotypes were determined and it was verified with χ^2 test whether their observed distributions

corresponded to the expected ones (according to the Hardy-Weinberg law). The statistical analysis also included a search for associations between *DRB3*/*Hae*III polymorphism and SCC in milk. Only genotypes found in at least 10 individuals were included in the analysis. Parity, season, stage of lactation, year of study and cow (random factor nested in *DRB3*/*Hae*III genotype as genetic background) were also considered as sources of variability. The year of animal's birth was not considered as a factor as it corresponded to the lactation number in the subsequent years of the study. Year was divided into four seasons: Winter-from December to February, spring-from March to May, summer-from June to August and autumn-from September to November. Lactation stage was set as: Stage I-from the 1st to the 4th month, stage II-from the 5th to the 8th month, stage III-the 9th and subsequent months. Lactation number 5 and the subsequent ones were treated as one category, mainly due to the decreasing number of available cows of older age and the observed tendency of increasing SCC in milk in later lactations. SCC in milk was transformed into a logarithmic scale to obtain normal distribution of this trait.

The results of the analyses were processed statistically according to STATISTICA data analysis software system, version 7.0, with multiple-factor mixed nested model. Means and standard deviations for different levels of the studied factors were also calculated. The significance of differences between the means was verified with the Duncan test.

The effect of *DRB3/Hae*III genotypes on daily milk yield and fat and protein content in milk in the year 2002 was also analysed. The influence of cow, season, parity and stage of lactation was considered. The model used here was analogous to the one described for the analysis of the effect of *DRB3/Hae*III on SCC in milk.

RESULTS AND DISCUSSION

In the studied herd of dairy cows, four *DRB3*/*Hae*III alleles-A, B, D and E were observed. The RFLs obtained by cutting the 284 bp PCR product were as follows: A-167, 65, 52; B-219, 65; D-190, 65, 29; and E-112, 87, 85. Their frequencies were as follows: A-0.560; B-0.195; D-0.008 and E-0.236. The alleles controlled the occurrence of eight out of ten possible genotypes. The results are presented in Table 1.

The RFLP of *DRB3* gene was studied by Van Eijk *et al.* (1992). They identified 6 alleles cut with *Hae*III restrictase into the following fragments: a-167, 65, 52; b-219, 65; c-167, 65, 49; d-190, 65, 29; e-167, 117 and f-167, 65, 48, 4 bp. The subsequent alleles: g-164, 65, 55; h-167, 65, 46, 6 and I-167, 113, 4 were identified by Gelhaus *et al.* (1995).

The frequencies of *DRB3*/*Hae*III alleles were studied by Miretti *et al.* (2001). In this *locus*, they identified four alleles: A, B, D and E, which determined ten possible genotypes: AA, BB, DD, EE, AB, AD, AE, BD and DE. The frequencies of the identified alleles were as follows: A-0,496; B-0.293; D-0.014 and E-0,224. Thus, these results are similar to those obtained in this study.

The fact that no alleles C, F, G, H and I were found in the studied population may result from their generally law frequency reported by other researchers in various breeds of cows. For example, Gilliespie *et al.* (1999) had found no alleles C, F, G, H and I in Jersey cows and similarly Miretti *et al.* (2001) had found no such alleles in seven populations of South American cattle.

Statistically significant difference was found in the studied population between the observed DRB3/*Hae*III genotype distribution and the expected one estimated according to the Hardy-Weinberg law. The number of observed BB and AE genotypes was higher than that expected. On the other hand, there were fewer EE, AB and BE genotypes compared with their expected numbers.

In this study, associations between ln SCC and *DRB3*/*Hae*III genotype, parity, season, lactation stage, year of study and cow (nested in *DRB3*/*Hae*III genotype) were investigated. The study included only genotypes with a frequency higher than 1%. Statistically significant associations were found between ln SCC and all the studied factors. The results are presented in Table 2.

Mean values for ln SCC, their standard deviations as well as the significance of differences between the mean values are presented in Table 3. The highest ln SCC (transformed into a logarithmic scale) was recorded in milk of AE-genotype cows (5.303), whereas the lowest ln SCC was observed in BB-genotype cows (4.867).

The results obtained in this study confirmed the proposition that BoLA-*DRB3* can be used as a marker of somatic cell concentration in milk and, in consequence, as a marker of susceptibility/resistance to mastitis in dairy cows. It was proved that there are associations between the allelic variants of the bovine MHC (BoLA) genes and the occurrence of disease and immune responsiveness in cattle (Xu *et al.*, 1993; Schukken *et al.*, 1994; Mallard *et al.*, 1995; Sharif *et al.*, 1998). For instance, Xu *et al.* (1993) found statistically significant associations between allelic variants of the class II BoLA-*DRB3* gene and resistance or susceptibility to persistent lymphocyotsis caused by the Bovine Leukemia Virus (BLV).

The effect of *DRB3* polymorphism on SCC in milk has been studied by many authors. Significant associations between SCC and various *DRB3* haplotypes and alleles were established by Oddgerisson *et al.* (1988), Mejdell *et al.* (1994), Schukken *et al.* (1994) and others. Sharif *et al.* (1998) studied *DRB3* polymorphism in 835 Holstein

Table 1: Frequencies and distribution of *DRB3* genotypes in the analysed herd

DRB3 genotype	Observed no. of cows	Observed frequency [%]	Expected no. of cows	Expected frequency [%]	obs.-exp.	(obs.-exp.)^
AA	60	32.9670	57.1648	31.410	2.8352	0.1406
BB	20	10.9890	6.9244	3.800	13.0756	24.6908
DD	1	0.5495	0.0125	0.010	0.9877	78.9091
EE	7	3.8462	10.1593	5.580	-3.1593	0.9825
AB	21	11.5385	39.7912	21.860	-18.791	8.8740
AD	1	0.5495	1.6813	0.920	-0.6813	0.2761
AE	62	34.0659	48.1978	26.480	13.8022	3.9525
BD	0	0.0000	0.5852	0.320	-0.5852	0.5852
BE	10	5.4945	16.7747	9.220	-6.7747	2.7361
DE	0	0.0000	0.7088	0.390	-0.7088	0.7088
Total	182	100.0000	182.0000	100.0000	0.0000	121.8556

Chi-square = 121.8556, df = 9, p < 0.000000

Table 2: Associations between ln SCC and the analysed factors

Source of variability	Degree of freedom	F-statistics	Probability
DRB3 genotype	4	4.01804	0.002982
Parity	4	7.33075	0.000013
Season	3	9.12568	0.000018
Stage of lactation	2	15.04959	0.000000
Year of study	3	5.30452	0.001308
Cow (nested factor in *DRB3* genotype)	173	5.39167	0.000000

Table 3: Means and standard deviations of ln SCC in milk related to the *DRB3* genotype

Factor	No.	Mean of ln SCC	Standard deviation	Significance of differences P = 0.01
1. *DRB3* AA	750	5.213	1.167	2, 5
2. *DRB3* BB	278	4.865	0.921	1, 3, 4
3. *DRB3* AB	297	5.162	1.083	2, 5
4. *DRB3* AE	899	5.303	1.168	2, 5
5. *DRB3* BE	147	4.928	1.155	1, 3, 4
Total	2371	5.182	1.141	

cows and 66 Jersey cows. They found no associations between *DRB3* alleles and Somatic Cell Score (SCS) in milk of Jersey cows. However, they established significant associations between *DRB3.2*16* alleles and low SCS in Holstein cows. The fact that no such association was found in Jersey cows might have resulted from the small number of cows in the herd and consequently a low infection incidence rate. Associations between *DRB3.2*16* allele and decreased SCC were also proved by Aarestrup *et al.* (1995) and Ashwell *et al.* (1996). Dietz *et al.* (1997a), on the other hand, found that *DRB3.2*16* allele had a significant effect on higher SCC in milk whereas Kelm *et al.* (1997) associated allele **23* with decreased SCC. A different result was obtained by Sharif *et al.* (1998), who found significant associations between allele **23* and increased SCC.

DRB3 polymorphism recognized by three restriction enzymes (*Rsa*I, *Bst*YI and *Hae*III) is characterized by a large number of gene variants. This corresponds to the actual variability within *DRB3* gene but makes it difficult to indicate the correct marker. This great variability is likely to be the reason for contradictory results of studies on associations between various *DRB3* alleles and resistance to udder infections. For example, Dietz *et al.* (1997a) and Kelm *et al.* (1997) associated allele **16* with a higher SCC whereas Starkenburg *et al.* (1997) and Sharif *et al.* (1998)-with a lower SCC. Moreover, Kelm *et al.* (1997) and Sharif *et al.* (2000) reported more cases of Clinical Mastitis (CM) in cows with allele **8* whilst Starkenburg *et al.* (1997) reported fewer cases of CM is such cows. What is more, great differences in *DRB3* allele frequencies are even found within one particular breed. For instance, in Jersey cows, allele **7* was found to be most frequent by Sharif *et al.* (1998) but was not found at all by Gilliespie *et al.* (1999). Therefore, it seems reasonable to study a single polymorphism recognized by only one restriction enzyme. *DRB3* gene variants identified in this way do not fully reflect the variability of this locus but they meet the criteria of a good marker.

The results presented in Table 4 shows associations between daily milk yield and fat and protein content and the analyzed factors, namely *DRB3/Hae*III genotype, parity, season, lactation stage and cow. No statistically significant association was found between the studied traits and *DRB3/Hae*III genotype.

Furthermore, no statistically significant association was found between the studied milk production traits and *DRB3/Hae*III genotype in this study. Associations between *DRB3* polymorphism and milk utility traits have also been studied by many authors. Sharif *et al.* (1999) evaluated possible relationships between BoLA-*DRB3* alleles and production traits, namely 305-day lactation milk yield and fat and protein yield, in a population of Canadian dairy cows (Holstein, n = 835 and Jersey, n = 66) over the course of two lactations. No significant associations were found between BoLA alleles and the above-mentioned production traits in Jerseys. In Holsteins, no associations were found between alleles *16 and *23 and the production traits but allele *8 was significantly associated with increased 305-day lactation milk, fat and protein yields. Allele *22, on the other hand, was associated with a lower milk and protein yield. Machado *et al.* (2005) studied associations between BoLA-*DRB3.2* alleles identified by PCR-RFLP and milk production in Gir breed. Two BoLA-*DRB3* alleles (**16* and **29*) were found to be significantly associated with milk production, suggesting that BoLA-*DRB3.2* locus

Table 4: Associations between daily milk yield, fat and protein content and the analysed factors

Factor	Daily milk yield probability	Significance	Fat content probability	Significance	Protein content probability	Significance
DRB3 genotype	0.069745	n.s.	0.796472	n.s.	0.389171	n.s.
Parity	0.000000	***	0.066358	n.s.	0.016039	*
Season	0.000000	***	0.000000	***	0.000000	***
Stage of lactation	0.000000	***	0.000000	***	0.000000	***
Cow (nested in *DRB3* genotype)	0.000000	***	0.000000	***	0.000000	***

*P = 0.05; ***P = 0,001; n.s.-non significant

itself or a linked QTL influences milk yield in 305-day lactation of Gir cows. Associations between BoLA-*DRB3* locus and economically important traits in Holstein cows were studied by Vukasinovic *et al.* (1997). Statistically significant association was found between BoLA-*DRB3* locus and PTA for milk, fat and protein yield and fat percentage, which indicates that either *DRB3* locus itself or a linked QTL influences these traits.

CONCLUSION

The results obtained in this study, confirm the hypothesis of using *DRB3* as a marker for SCC and, what follows, for resistance/susceptibility to mastitis in dairy cows. However, further studies are necessary to confirm these results before *DRB3* is be used as a SCC marker in mass selection of dairy cattle.

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