Animal Science Papers and Reports vol. 26 (2008) no. 1, 37-48
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

An association of BoLA alleles *DRB3.2*16* and *DRB3.2*23* with occurrence of *mastitis* caused by different bacterial species in two herds of dairy cows*

Karima Galal Abdel Hameed[1], Grażyna Sender[2], Agnieszka Korwin-Kossakowska[2*]**

[1] South Valley University, Qena, Egypt

[2] Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland

(Received November 15, 2007; accepted January 22, 2008)

The objective of the study was to identify an association between the genotypes of *BoLA-DRB3.2 loci* (16/-* and **23/-*) and occurrence of sub-clinical *mastitis* or latent mammary gland infections caused by different bacterial species in dairy cows. Blood was withdrawn from of 275 cows from two Polish dairy herds, and animals were genotyped for *BoLA-DRB3.2*16* and *BoLA-DRB3.2*23* using MPT-PCR technique. The bacteriological status of the mammary gland was determined by collecting and culturing composite milk samples. SCC was recorded by Fossomatic device after bacteriological examination. No relationship was identified between cows of **16/-* or **23/- Bola-DRB3.2* genotype in susceptibility/resistance to sub-clinical *mastitis* caused by *Staphylococcus aureus*. However, genotype **23/-* was found to be associated (P≤0.01) with increased susceptibility to sub-clinical *mastitis* caused by *Streptococcus dysgalactiae*.**

KEY WORDS: BoLA genes / mastitis / dairy cattle / *Staphylococcus aureus* / *Streptococcus dysgalactiae*/ coagulase negative staphylococci

Mastitis is a difficult problem to comprehend as it is caused by many factors acting simultaneously. Microorganisms of different species are responsible for the infection. However, for bacteria to enter the mammary gland and establish themselves

*Supported by the State Committee for Scientific Research Project 2 PO6D 03026 and Polish Academy of Sciences Institute of Genetics and Animal Breeding, Project SI-1.7.
**Corresponding author: g.sender@ighz.pl

to reach the infection point a multitude of non-microbial factors may be involved: hygiene, housing, climate, milking machines, feed and genetic structure of cows. It is even more difficult to generalize about the relative importance of each factor, as certain factors affect certain microorganisms in particular. There are three important defence mechanisms of the udder. The first is closure of the teat canal that efficiently prevents pathogens from entering the teat cystern. Thus, to keep the teat canal in a good condition, it is important to use a proper milking technique. The second factor is efficient immune response, weakened by stress, malnutrition, poor quality feed and water [Hallén-Sandgren *et al*. 1997]. The third that decreases the risk of *mastitis* is washing out of pathogens at milking time [Klastrup *et al*. 1987].

A large number of organisms have been identified as causing bovine *mastitis*. They can be classified as either major or minor pathogens. The major and minor pathogens can further be subdivided into organisms causing contagious intramammary infection and agents from environmental sources [Radostits *et al*. 1994].

Contagious *mastitis* is defined as intramammary infection transmitted directly from cow to cow [Erskine 2001]. The most common contagious organisms are *Staphylococcus aureus (S. aureus), Streptococcus agalactiae (Str. agalactiae), Corynebacterium bovis* and *Mycoplasma bovis*. They are spread from infected quarters to healthy quarters of another cow. Contagious microorganisms are well adapted to survive in the udder and usually establish mild clinical infections of long duration (chronic infections).

S. aureus has emerged as one of the most prevalent contagious *mastitis*-causing pathogens that colonize the teats when there is damage to the skin surface. Transmission occurs mainly through contaminated milking machines and udder wash clothes, as well as the hands of machine operators. The organism can survive outside the cow for a short time only [Risco *et al.* 1999]. Most often, infections caused by *S. aureus* are sub-clinical in nature with periodic flare-up of clinical symptoms [Bramley and Dodd 1984]. Staphylococcal *mastitis* is characterized by its irregular shedding pattern that complicates diagnosis. Some cows never shed the organisms from the udders, others shed the staphylococci intermittently at short intervals, while still others show a persistent shedding state, extended over several lactation periods. Fighting *S. aureus* in a herd requires a systematic programme which can be summed up in sampling, culling, grouping and dry cow therapy [Ahlner 2003].

The main environmental organisms are gram-negative bacteria (mostly coliforms) and environmental streptococci. The gram-negative bacteria include *Escherichia coli (E. coli), Klebsiella sp., Enterobacter sp., Citrobacter sp., Seratia, Pseudomonas sp.* and *Proteus*. The environmental streptococci include *Streptococcus uberis (Str. uberis), Streptococcus dysgalactiae (Str. dysgalactiae)*, and *Streptococcus equinus (Str. equinus)*. Intramammary infections with these pathogens are frequent, but often of short duration, and usually result in clinical *mastitis* (CM) of different severity.

Str. dysgalactiae is one of the major pathogens, belonging to the Lancefield's group C, and is no longer included in the group of streptococci, but retained its name

within the *mastitis* field terminology [Buzalski and Seuna 1995]. The organism lives almost anywhere and its spread can be stopped by dipping the whole teat to the base of the udder. However, Sandholm and Payörälä [1995] reported that the incidence of *Str. dysgalactiae* increases in herds where teat dipping and dry cow therapy are applied. Buzalski and Payörälä (1995) showed that in herds infected with *Str. dysgalactiae* high cell counts in the bulk milk are observed. They found the infection with the organism to be related to teat lesions.

Coagulase-negative staphylococci (CNS) were previously called micrococci. Species most often isolated from CNS *mastitis* are *Staphylococcus hyicus (S. hyicus)*, *Staphylococcus simulans (S. simulans)*, *Staphylococcus epidermidis (S. epidermidis)*, *Staphylococcus warneri (S. warneri)*, *Staphylococcus xylosus (S. xylosus)*, *Staphylococcus hominis (S. hominis)* and *Staphylococcus haemolyticus (S. haemolyticus)* [Buzalski and Seuna 1995]. *Mastitis* caused by these pathogens occurs at all stages of lactation but is most common during drying-off and soon after calving. *Mastitis* caused by CNS is also considered milder than that caused by *S. aureus,* since the former is less virulent. CNS bacteria can often cause teat infection leading to only a slight increase in milk cells count whereas *mastitis* occurs particularly in heifers. Pankey *et al.* [1996] stated that CNS were isolated from 21.8% of heifer udders. A study conducted in USA by Trinitad *et al*. [1990] showed that up to 90% of heifers' quarters are infected already before parturition and 70% of them are infected with CNS. Laevens *et al.* [1997] concluded that isolation of CNS has resulted in significant increase in somatic cell count (SCC) with least squares mean (log-transformed) of 3.97.

Genes encoding the major histocompatibility complex (MHC) are attractive candidate genes for the associations with disease resistance and susceptibility, because of the MHC role in immune response [Hameed *el al.* 2006]. The MHC, known as the bovine leukocyte antigen (BoLA) region in cattle, is a tightly linked cluster of genes, spanning over 2.5 Mb of the cattle genome. The majority of the MHC gene products are associated with host defence and intercellular communication [Lewin 1996]. Many of the MHC molecules function as antigen- presenting structures. Consequently, the particular set of MHC molecules expressed by an individual affects its response to antigens of infectious organisms. MHC therefore, has been implicated in susceptibility and resistance to infectious diseases and in the development of autoimmunity [Lewin 1996]. In cattle, associations have been found between BoLA alleles and diseases such as dermatophilosis [Maillard *et al.* 1999], decreased risk of cystic ovarian disease and retained placenta [Sharif *et al.* 1998], posterior spinal paresis [Park *et al.* 1993], resistance to persistent lymphocytosis caused by bovine leukaemia virus [Xu *et al.* 1993] and *mastitis* [Schukken *et al.* 1994, Sharif *et al.* 1998].

The *BoLA-DRB3* gene region was studied extensively for associations with diseases because of its extremely wide polymorphism [Takeshima *et al*. 2002]. The exon 2 of *BoLA-DRB3* encodes the peptide-binding residues and is the most variable region of the class II alleles. As a result of high polymorphism in *BoLA-DRB3 locus*,

the *BoLA-DRB3.2* alleles potentially affect many traits related to immunity, SCC, and *mastitis* incidence. Therefore it is of particular interest to study the association between *BoLA-DRB3.2* alleles and occurrence of *mastitis* caused by specific pathogen.

In several studies the relationship was investigated between BoLA Class II alleles and different *mastitis* indicators [Dietz *et al.* 1997, Kelm *et al.* 1997, Starkenburg *et al.* 1997 , Sharif *et al.* 1998, 2000, Rupp *et al*. 2007]. There are many alleles of *BoLA-DRB3.2* associated with SCC and variety of them was found to be related to susceptibility and (or) resistance to CM. Most studies focused only on clinical *mastitis* (CM) and did not pay attention to pathogens causing *mastitis*. Dietz *et al.* [1997], Kelm *et al.* [1997], Starkenburg *et al.* [1997] and Sharif *et al.* [1998] have all demonstrated associations between BoLA allele **16* and SCC. These studies have produced conflicting results and suggested the need for further study on a larger population. The reason for conflicting results might be different pathogens causing *mastitis* in examined populations. It suggested the need for further study on particular pathogens causing *mastitis*.

The objective of this study was to explore the interrelation between the frequency of sub-clinical *mastitis* or latent udder infection incidence caused by different bacterial species as related to genotypes of *BoLA-DRB3.2 loci *16/-* and **23/-* in cattle, based upon records obtained from two dairy herds maintained in Poland.

Material and methods

During year 2004 a total of 722 composite milk samples were analysed from 275 lactating Polish Holstein cows kept in two herds (360 samples from 138 cows of herd K and 362 samples from 137 cows of herd P). The bacteriological status of the mammary gland was determined by collecting and culturing duplicate composite milk samples. A third sample was collected from each cow and cultured when the result for sample 1 differed from that for sample 2.

Composite milk samples were examined according to the recommendations by National Mastitis Council [Hogan *et al*. 1999]. Details of bacteriological examinations were those described by Hameed *at al*. (2007). SCC was recorded by Fossomatic 90 A/SN (FOSS ELECTRIC, Denmark) after bacteriological examination. The distinction between "normal" and "high" cell count of milk was based on Philpot and Nickerson [2006].

The bacterial isolates were assorted to seven species: *S. aureus*, CNS, *Str. agalactiae, Str. dysgalactiae, E. coli,* other coliforms (*Klebsiella* and *Enterobacter*) and other bacterial species (environmental streptococci, *Bacillus cereus*, *Corynebacterium species*, *Pseudomonas species*). The types of bacteria were classified into two groups, *i.e.* contagious (*S. aureus* or *Str. agalactiae)* and environmental (CNS, *Str. dysgalactiae, E. coli,* other coliforms and other bacteria).

The diagnosis of sub-clinical *mastitis* and latent infections of the examined milk samples was done in accordance with the criteria given below.

1. A sample was classified as indicating contagious or environmental sub-clinical *mastitis* when there were ≥500 colony forming units (cfu)/ml or ≥2000 cfu/ml of the bacterial species, respectively, and SCC was $\geq 100 \times 10^3$ cells/ml.
2. Latent contagious or environmental infections were diagnosed when there were ≥500 cfu/ml or ≥2000 cfu/ml of the bacterial species, respectively, and SCC was $<100 \times 10^3$ cells/ml.

The diagnosis of sub-clinical *mastitis* and latent infections of cows during lactation (examined two to three times) was done in accordance with the criteria given below.

1. Lactating cows were classified as afflicted by sub-clinical contagious *mastitis* when two milk samples produced ≥500 cfu/ml or three milk samples produced ≥100 cfu/ml of the same contagious pathogen, and SCC was $\geq 100 \times 10^3$ cells/ml.
2. Sub-clinical environmental *mastitis* was diagnosed when two milk samples produced ≥2000 cfu/ml or three milk samples produced ≥400 cfu/ml of the same environmental pathogen and SCC were $\geq 100 \times 10^3$ cells/ml.
3. Latent infections were diagnosed when two or three milk samples showed $SCC < 100 \times 10^3$. The classification of a cow as afflicted by contagious and environmental *mastitis* followed the same criteria as those used for classification of contagious and environmental sub-clinical *mastitis*.

Blood of 275 cows was withdrawn to determine of *BoLA-DRB3.2*16* and **23* using MPT-PCR technique [Ledwidge *et al.* 2001].

The DNA amplification was performed using primers suggested by Ledwidge *et al*. [2001]. Two alleles have been analysed using the MPT-PCR method in which four primers were used − two outer primers to amplify exon 2, and two inner to amplify the specific alleles simultaneously. The PCR reaction was carried out in GenAmp PCR System 9600 Thermal Cycler (AB) using the following cycling parametres: 95°C for 15 min 30 cycles (95°C for 30 s, 56°C for 90 s, and 72°C for 90 s) followed by 72°C for 10 min.

The PCR products were visualized using 2.5% agarose gel. The use of four MPT-PCR primers generated three possible fragments: a 395 bp fragment of exon 2 in all animals, a 151 bp fragment for the animal carrying allele **16* and a 196 bp fragment for the animal carrying the allele **23*. The 395 bp fragment also served as an internal control for the PCR.

Analysis of variance was used (GLM procedure of SAS) to estimate the associations between *BoLA-DRB3.2* genotype, sub-clinical *mastitis* and latent infection caused by all examined bacterial species (pooled), and separately for bacteria of higher prevalence (*S. aureus*, CNS, *Str. dysgalactiae)*. The data were dichotomous (1=yes, zero=no) with "1" representing at least one incidence of *mastitis* in the denoted period. The statistical analysis was made separately for individual milk samples as preliminary calculation and final evaluation for cows diagnosed during lactation.

The model used for the preliminary evaluation of probability of latent infection, and sub-clinical *mastitis* caused by different bacterial species was accounted for the

(*BoLA-DRB3.2* genotype (**16/-*, **23/-*, **16/23* and *-/-*), herd, interaction between animals' genotype and herd, repetition (repetition 1=275 cows, repetition 2=243 cows and repetition 3=201 cows), and season of examination (summer or autumn).

The statistical model used for final evaluation of probability of latent infection, and sub-clinical *mastitis* for cows diagnosed during lactation was accounted for *BoLA-DRB3.2* genotype (**16/-*, **23/-*, **16/23* and *-/-*), herd, and interaction between herd and genotype.

Results and discussion

Table 1 show the LSMs of probability of latent infections caused by different bacterial species (pooled), *S. aureus,* and coagulase-negative staphylococci (CNS) in the milk samples examined in relation to the *BoLA-DRB3.2* genotype. The higher prevalence of latent infections caused by CNS in animals of **16/-* genotype was observed, followed by animals of **23/-* genotype. LSMs of prevalence for latent CNS infections in animals assumed as having other alleles were found different (P≤0.05) from those of genotype **16/-*. Unfortunately, prevalence of latent infections of examined milk samples was very low. Due to low number of *Str. dysgalactiae* latent infections (three milk samples only) these data were not presented.

Table 1. BoLA-*DRB3* genotype effect on the prevalence of latent infection caused by different bacterial species (pooled), *S. aureus*, and coagulase-negative *staphylococci* in the examined samples of milk

BoLA-*DRB3* genotype		Different bacterial species (pooled)	*S. aureus*	Coagulase-negative *staphylococci*
	n	22	1	5
**16/-*	LSM	0.11	0.04	0.04[a]
	SE	0.03	0.02	0.01
	n	8	1	2
**23/-*	LSM	0.06	0.01	0.02
	SE	0.04	0.03	0.02
	n	1	0	0
**16/23*	LSM	0.01	-0.12	0.00
	SE	0.08	0.06	0.03
	n	50	16	4
- /-	LSM	0.04	0.02	0.01[a]
	SE	0.02	0.01	0.01

n – number of samples.
[a]Means bearing the same superscripts differ significantly at P≤0.05.

In Table 2 LSMs of probability of sub-clinical *mastitis* caused by different bacterial species (pooled), *S. aureus*, CNS and *Str. dysgalactiae* in the preliminary analysis are shown across the *BoLA-DRB3.2* genotypes. It is clear that *BoLA-DRB3.2* genotype was associated (P≤0.01) only with occurrence of sub-clinical *mastitis* caused by *Str.*

Table 2. BoLA-*DRB3* genotype effect on the prevalence of sub-clinical *mastitis* caused by different bacterial species (pooled, *S. aureus* and coagulase-negative *staphylococci* and *Str. dysgalactiae* in the examined samples of milk

BoLA-*DRB3* genotype		Different bacterial species (pooled)	*S. aureus*	Coagulase-negative *staphylococci*	*Str. dysgalactiae*
	n	48	18	16	2
**16/-*	LSM	0.38	0.15	0.12	0.01^{A}
	SE	0.04	0.03	0.02	0.02
	n	26	5	6	8
**23/-*	LSM	0.41	0.10	0.10	0.11^{aAB}
	SE	0.06	0.04	0.03	0.02
	n	8	3	0	0
**16/23*	LSM	0.52	0.20	0.00	-0.00^{a}
	SE	0.12	0.10	0.07	0.05
	n	210	71	39	21
- /-	LSM	0.43	0.15	0.10	0.03^{B}
	SE	0.03	0.02	0.01	0.01

n – number of samples.
[a]Means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

dysgalactiae, although *S. aureus* and CNS are the main causes of sub-clinical udder inflammation and *Str. dysgalactiae* seldom contributes to its sub-clinical form. Allele **23* was found to be associated with the increase in *Str. dysgalactiae* sub-clinical *mastitis* prevalence. Moreover, LSMs of prevalence in animals of **23/-* genotype differed from those carrying **16/-* genotype (P≤0.01), or carrying remaining alleles (P≤0.01), as well as from heterozygous animals (P≤0.05).

The probability of latent infection in cows diagnosed during lactation as related to the *BoLA-DRB3.2* genotype is summarized in Table 3. The genotype was found significantly associated with neither different bacterial species (pooled) nor *S. aureus* infections, but showed the relation with latent infection caused by CNS. Due to low number of *Str. dysgalactiae* latent infections (one cow diagnosed during lactation) these data were not presented. An elevated prevalence of latent infection caused by CNS in animals of genotype **16/-* was found differing (P≤0.01) from the cows carrying the other alleles. Unfortunately, prevalence of latent infections in the cows diagnosed during lactation was very low.

In Table 4 LSMs of probability of sub-clinical *mastitis* in the cows diagnosed during lactation are shown across the *BoLA-DRB3.2* genotypes effects. The results indicate that *BoLA-DRB3.2* genotype affected (P≤0.01) only the prevalence of sub-clinical *mastitis* attributed to *Str. dysgalactiae*. LSMs of *Str. dysgalactiae* sub-clinical *mastitis* prevalence in animals carrying allele **23* differed (P≤0.01) from those carrying **16* and carrying the other alleles.

Table 3. BoLA-*DRB3* genotype effect on the prevalence of latent infection caused by different bacterial species (pooled), *S. aureus*, and coagulase-negative *staphylococci* in the cows diagnosed during lactation

BoLA-*DRB3* genotype		Different bacterial species (pooled)	*S. aureus*	Coagulase-negative *staphylococci*
	n	7	2	3
**16/-*	LSM	0.18	0.05	0.07[A]
	SE	0.06	0.02	0.02
	n	2	0	1
**23/-*	LSM	0.07	0.00	0.03
	SE	0.07	0.03	0.03
	n	1	0	0
**16/23*	LSM	0.25	-0.00	-0.00
	SE	0.16	0.08	0.07
	n	18	4	1
- /-	LSM	0.11	0.03	0.00[A]
	SE	0.03	0.01	0.01

n – number of samples.
[a]Means bearing the same superscripts differ significantly at P≤0.01.

Table 4. BoLA-*DRB3* genotype effect on the prevalence of sub-clinical *mastitis* caused by different bacterial species (pooled, *S. aureus* and coagulase-negative *staphylococci* and *Str. dysgalactiae* in the cows diagnosed during lactation

BoLA-*DRB3* genotype		Different bacterial species (pooled)	*S. aureus*	Coagulase-negative *staphylococci*	*Str. dysgalactiae*
	n	14	6	5	0
**16/-*	LSM	0.35	0.15	0.12	0.00[A]
	SE	0.07	0.06	0.04	0.03
	n	10	3	1	4
**23/-*	LSM	0.50	0.16	0.03	0.18[AB]
	SE	0.10	0.07	0.06	0.04
	n	1	1	0	0
**16/23*	LSM	0.25	0.25	0.00	-0.00
	SE	0.23	0.18	0.13	0.10
	n	63	26	13	7
- /-	LSM	0.39	0.16	0.08	0.04[B]
	SE	0.03	0.03	0.02	0.01

n – number of samples.
[a]Means bearing the same superscripts differ significantly at P≤0.01.

In the present study, two different analyses involving all infections were conducted and each provided different information. The first analysis (preliminary one and summarized in Tab. 1 and 2) provided information about how the genotype affects the occurrence of different types of *mastitis* caused by different bacteria in the examined milk samples. The second analysis (final one and summarized in Tab. 3 and 4) involved the same criteria, but on the level of cows that were diagnosed as *mastitis*-afflicted during lactation. This means that comparative effect of genotype reflects how the genotype affected the occurrence of different types of *mastitis* caused by different bacteria. While several studies have attempted at investigating associations between *BoLA-DRB3.2* alleles and clinical *mastitis* [Kelm *et al.* 1997, Sharif *et al.* 1998, 2000], there is no study available, so far, being devoted to the sub-clinical or latent udder inflammation caused by particular bacterial species. So the present study was designed to develop a better understanding of associations between the *BoLA-DRB3.2* alleles and different types of *mastitis*.

In this study, genotype **16/-* was found associated significantly with increased susceptibility to latent CNS infection as shown in both types of analysis – preliminary ($P \leq 0.05$) and final ($P \leq 0.01$), respectively. Unfortunately, due to low number of latent infection cases in data examined, drawing any conclusions is not possible. Curiously, allele **16* was the most common allele (frequency 0.10) in this study. This outcome is in accordance with Dietz *et al*. [1997] who concluded that animals of genotype **16/-* were more susceptible to intramammary infection. This is not in accordance with the results obtained by Sharif *et al.* [2000] in that cows carrying allele **23* were found more susceptible to clinical *mastitis* caused by *Staphylococcus sp.* However, in the present study, genotype **23/-* was associated ($P \leq 0.01$) with increased susceptibility to sub-clinical *mastitis* caused by *Str. dysgalactiae* in both milk samples examined and in cows diagnosed during lactation. This is in accordance with Sharif *et al.* [1998] who found the association between genotype **23/-* and increased susceptibility to clinical coliform *mastitis*. The present study does, however, support the findings of Sharif *et al*. [1998]. There was important difference between the two field studies, in that the detection of clinical *mastitis* by Sharif *et al*. [1998] was dependent on the producer's ability to identify cases, while the detection of sub-clinical *mastitis* in the performed study was independent of the producer. For this reason, the results for sub-clinical *mastitis* may be more reliable than for its clinical type (CM).

No significant association was found in this study between any type of *mastitis* (latent, sub-clinical) caused by *S. aureus* and either alleles **16* or **23* in both types of analysis (preliminary and final).

Since *mastitis* is a multifactorial disease with a complex etiology, these discrepancies may be explained by the difference in causative organism, genetic background, micro-environmental factors, interactions between environment and background, genes and/or criteria set for diagnosis of the condition.

The statistical models were designed to take into account the effect of herd on occurrence of latent infection and sub-clinical *mastitis* in both preliminary and final

analysis. Herd was found to be associated (P≤0.01) with occurrence of sub-clinical *mastitis* caused by different bacterial species, as reflected in the examined milk samples. Owing to the fact that herd means environment, it was important to include herd and the interaction between herd and genotype in the statistical model to show the environmental effect. Data were corrected for the effect of environment.

Hypothetically, allele **23*, which was in association with occurrence of sub-clinical *mastitis* caused by *Str. dysgalactiae*, might lack efficiency in presenting antigens of common bacterial causative agents of *mastitis* to T-lymphocytes in order to generate specific immunity. Alternatively, the allele in question might indirectly affect the occurrence of *mastitis* through linked-gene effects. Noteworthy, an association between allele **23* and resistance to persistent lymphocytosis caused by bovine leukaemia virus has been detected by Xu *et al*. [1993] and further confirmed by Sulimova *et al.* [1995]. Hence, selection for resistance to one disease may favourably reduce or ultimately eradicate it from a population, but may also increase susceptibility to another disease, or adversely affect production traits. Based on this, the results presented here suggest that one genotype may enhance infection by certain bacterial species, but at the same time may resist infection by another.

It is quite important to note that the observation described here was the first made on Polish cattle population. Unfortunately, there were small numbers of animals having the relation between *BoLA-DRB3.2* genotype and *mastitis*, and especially the latent udder infections. So, the results of this study are presented as preliminary. Number of examined animals can be the reason for the unclear association between *BoLA-DRB3.2* genotype and different types of *mastitis*.

In summary, it should be stated that no association between cows carrying allele **16* and **23* was identified with susceptibility/resistance to sub-clinical *S. aureus* or CNS *mastitis*. However, genotype **23/-* was found related (P≤0.01) to increased susceptibility to sub-clinical *mastitis* caused by *Str. dysgalactiae*.

REFERENCES

1. AHLNER S., 2003 – Prevalence of sub-clinical mastitis in Uruguay. A project degree, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Uppsala University, 10-11.
2. BRAMLEY A.J., DODD F.H., 1984 – Reviews of the progress of dairy science: mastitis control progress and prospects. ***Journal of Dairy Research*** 51, 481-512.
3. BUZALSKI T.H., PAYÖRÄLÄ S., 1995 – The bovine udder and mastitis: Monitoring and management of udder health at the farm. University of Helsinki, Faculty of Veterinary Medicine ISB: 951-834-047-1, 252-260.
4. BUZALSKI T.H., SEUNA E., 1995 – The bovine udder and mastitis: Isolation and identification of pathogens from milk. University of Helsinki, Faculty of Veterinary Medicine ISBN: 951-834-047-1, 121-141.
5. DIETZ A.B., COHEN N.D., TIMMS L., KEHRLI M.E., 1997 – Bovine lymphocyte antigen class II alleles as risk factors for high somatic cell counts in milk of lactating dairy cows. ***Journal of Dairy Science*** 80, 406-412.
6. ERSKINE R.J., 2001 – Mastitis control in dairy herds. In: Radostits O.M. (2nd edition) Herd Health. Food Animal Production Medicine. W.B. Saunders Company, Pennsylvania, USA, 397-433.

7. HALLÉN-SANDGREN C., SVENSSON C., TIVEMO M., EMANUELSON U., 1997 – Riskfaktorer för juverinflammation, Sveriges lantbruksuniversitet, Uppsala. ***Fakta Husdjur*** 13, 4s.
8. HAMEED K.G.A, SENDER G., MAYNTZ M., 2006 – Major histocompatibility complex polymorphism and *mastitis* resistance – a review. ***Animal Science Papers and Reports***, 24(1) 11-25.
9. HAMEED K.G.A, SENDER G., KORWIN-KOSSAKOWSKA A., 2007 – Public health hazard due to mastitis in dairy cows**.** ***Animal Science Papers and Reports***, 25(2) 73-85.
10. HOGAN J.S., GONZALEZ R.N., HARMON R.J., NICKERSON S.C., OLIVER S.P., PANKEY J.W., SMITH K.L., 1999 – Laboratory and field handbook on bovine mastitis, Revised edition. National Mastitis Council, Inc., Wisconsin, Madison, 1-33.
11. KELM S.C., DETILLEUX J.C., FREEMAN A.E., KEHRLI M.E., DIETZ A.B., FOX L.K., BUTLER J.E., KASCKOVIES D.H., KELLEY 1997 – Genetic association between parameters of innate immunity and measures of mastitis in periparturient Holstein cattle. ***Journal of Dairy Science*** 80, 1767-1775.
12. KLASTRUP O., BAKKEN G., BRAMLEY J., BUSHNELL R., 1987 – Environmental influences on bovine mastitis. ***Bulletin of the International Dairy Federation*** 227, 1-37.
13. LAEVENS H., DELUYKER H., SCHUKKEN Y.H., DE MEULEMEESTER L., VANDERMEERSCH R., DE MUELENAERE E., DE KRUIF A., 1997 – Influence of parity and stage of lactation on somatic cell count in bacteriologically negative dairy cows. ***Journal of Dairy Science*** 80, 3219-3226.
14. LEDWIDGE S.A., MALLARD B.A., GIBSON J.P., JANSEN G.B., JIANG Z.H., 2001 – Multi-primer target PCR for rapid identification of bovine DRB3 alleles. ***Animal Genetics*** 32, 219-221.
15. LEWIN H.A., 1996 – Genetic organization, polymorphism and function of the bovine major histocompatibility complex. In: The major histocompatibility complex region of domestic animal species (L.B.Schook and S.J. Lamont, eds.) CRC Press, Boca Raton, Florida 65-98.
16. MAILLARD J.C., RENARD C., CHARDON P., CHANTAL I., BENSAID A., 1999 – Characterisation of 18 new *BoLA-DRB3* alleles. ***Animal Genetics*** **30,** 200-203.
17. PANKEY J.W., PANKEY P.B., BARKER R.M., WILLIAMSON J.H., WOOLFORD M.W., 1996 – The prevalence of mastitis in primiparous heifers in eleven Waikato dairy herds. ***New Zealand Veterinary Journal*** 44, 41-44.
18. PARK C.A., HINES H.C., MONKE D.R., THRELFALL W.T., 1993 – Association between the bovine major histocompatibility complex and chronic posterior spinal paresis – a form of ankylosing spondylitis – in Holstein bulls. ***Animal Genetics*** 24, 53-58.
19. PHILPOT W.N., NICKERSON S.C., – 2006 – Zwyciężyć w walce z mastitis (To win the mastitis) In Polish. Scientific edition Malinowski E., Westfalia Surge Inc, ISBN 3-923637-0-1, 1-189.
20. RADOSTITS O.M., LESLIE K.E., FETROW J., 1994 – Herd Health. In: Food Animal Production Medicine. W.B. Saunders Company Ltd., London, United Kingdom,. 229-273.
21. RISCO C.A., DONOVAN G.A., HERNANDEZ J., 1999 – Clinical mastitis associated with abortion in dairy cows. ***Journal of Dairy Science*** 82, 1684-1689.
22. RUPP R., HERNANDEZ A., MALLARD B.A. 2007 – Association of bovine leukocyte Antigen (BoLA) DRB3.2 with immune response, mastitis, and production and type traits in Canadian Holsteins. ***Journal of Dairy Science*** 90, 1029-1038.
23. SANDHOLM M., PAYÖRÄLÄ H., 1995 – The bovine udder and mastitis: Antibacterial defence mechanisms of the udder. University of Helsinki, Faculty of Veterinary Medicine ISBN: 951-834-047-1, 37-48.
24. SCHUKKEN Y.H., MALLARD B.A., DEKKERS J.C.M., LESLIE K.E., STEAR M.J., 1994 – Genetic impact on the risk of intramammary infection following *Staphylococcus aureus* challenge. ***Journal of Dairy Science*** 77, 639-647.

25. SHARIF S., MALLARD B.A., SARGEANT J.M., 2000 – Presence of glutamine at position 74 of pocket 4 in the BoLA-DR antigen binding groove is associated with occurrence of clinical mastitis caused by *Staphylococcus species*. ***Veterinary Immunology and Immunopathology*** 76, 231-238.
26. SHARIF S., MALLARD B.A., WILKIE B.N., SARGEANT J.M., SCOTT H.M., DEKKERS J.C.M., LESLIE K.E., 1998 – Association of the bovine major histocompatibility complex DRB3 (*BoLA-DRB3*) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. ***Animal Genetics*** 29, 185-193.
27. STARKENBURG R.J., HANSEN L.B., KEHRLI M.E., CHESTER-JONES H., 1997 – Frequencies and effects of alternative DRB3.2 alleles of bovine lymphocyte antigen for Holsteins in milk selection and control lines. ***Journal of Dairy Science*** 80, 3411-3419.
28. SULIMOVA G.E., UDINA 1.G., SHAIKHAEV G.O., ZAKHAROV L.A., 1995 – DNA polymorphism at the *BOLA-DRB3* gene of cattle in relation to resistance and susceptibility to leukaemia. ***Russian Journal of Genetics*** 31, 1105-1109.
29. TAKESHIMA S., NAKAI Y., OHTA M., AIDA Y., 2002 – Characterization of DRB3 alleles in the MHC of Japanese Shorthorn Cattle by polymerase chain reaction-sequence-based typing***. Journal of Dairy Science*** 85, 1630-1632.
30. TRINIDAD P., NICKERSON S.C., ALLEY T.K., 1990 – Prevalence of intra-mammary infection and teat canal colonization in unbred and primigravid dairy heifers. ***Journal of Dairy Science*** 73, 107-114.
31. XU A., VAN EIJK M.J., PARK C., LEWIN H.A., 1993 – Polymorphism in the *BoLA-DRB3* exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukaemia virus. ***Journal of Immunology*** 151, 6977-6985.

Karima Galal Abdel Hameed, Grażyna Sender,
Agnieszka Korwin-Kossakowska

Związek genotypów w *locus BoLA-DRB3.2* (**16/-* i **23/-*) z występowaniem zapalenia wymienia wywołanego przez różne bakterie

Streszczenie

Celem pracy było zbadanie współzależności między podklinicznymi zapaleniami i utajonymi zakażeniami gruczołu mlekowego wywołanymi przez różne drobnoustroje a genotypami w *locus BoLA-DRB3.2 (*16/-* i **23/-)* krów mlecznych utrzymywanych w Polsce w dwóch stadach. W próbkach krwi pobranych od 275 krów zidentyfikowano metodą MPT-PCR allele **16* i **23.* Badanie bakteriologiczne wykonano w próbkach mleka zbiorczego z czterech ćwiartek wymienia. Liczbę komórek somatycznych określano za pomocą aparatu Fossomatic, po przeprowadzonych analizach bakteriologicznych. Nie udowodniono związku między nosicielstwem *allelu *16 i *23* przez krowy a ich podatnością/opornością na podkliniczne zapalenie wymienia wywołane przez gronkowca złocistego. Istotny natomiast okazał się związek wymienionych alleli (P≤0,01) z występowaniem podklinicznych zapaleń wymienia wywoływanych przez *Streptococcus dysgalactiae,* przy czym wzrost infekcji i podklinicznych zapaleń zależał od genotypu **23/-*.