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Clinical Mastitis and Combined Defensin Polymorphism in Dairy Cattle

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Abstract: Identification of marker sequences related to immunity towards mastitis may be instrumental in improving resistance against this trait and as a result may reduce the costs related to the prevention and treatment of the disease. The ideal candidate genetic markers for immunity towards mastitis are the genes encoding bovine defensins which belong to the wide and varied group of peptide antibiotics. A lot of antimicrobial peptides identified in cattle have been classified as β-defensins. Defensins are particularly active against gram-positive bacteria and fungi but at higher concentrations they are also, capable of destroying gram-negative bacteria, mycobacteria, enveloped viruses and some protozoons. The aim of this study was to search for associations between the occurrence of clinical mastitis and Combined Defensin Genotypes (CDG) and to investigate the possibility of using defensin gene polymorphisms in marker-assisted selection for immunity towards mastitis in dairy cows. This study included such indicators as the number of clinical cases of mastitis acuta and chronica, number of affected udder quarters and duration of the condition in 1,025 cows (Polish Holstein-Friesian breed) kept on a farm located in the North-Western region of Poland. The cows were of different ages and in different lactations parities (from 1st to 6th). An analysis of associations between selected CDGs and susceptibility/immunity towards mastitis has showed statistically significant relations with regard to all the indicators under study and CDGs. Moreover, some genotypes have been found to have different effects on chronic and acute infections.

Key words: β-defensin infection, clinical mastitis, dairy cow, chronic and acute infactions, genotypes

INTRODUCTION

One major problem in dairy cattle husbandry is the prevalence of udder infections. Despite the considerable technological progress in animal production, udder inflammation is still commonplace, particularly in high-yielding herds. Identification of marker sequences related to immunity towards mastitis may be instrumental in improving this trait and as a result may reduce the costs related to the prevention and treatment of the disease. It has been found that in the case of low-heritability traits such as immunity towards mastitis, Marker-Assisted Selection (MAS) or genomic selection produce better results compared with the conventional selection methods (Lande and Thompson, 1990). The primary focus of the search for candidate genes associated with the incidence of mastitis is to identify genes that are involved in the immune processes in the udder by examining the biological functions of their products and analysing the expression of selected genes. Researchers have already identified many candidate marker loci for mastitis (Pighetti and Elliott, 2011). The ideal candidate genetic markers for immunity to wards mastitis are the genes encoding bovine defensins which belong to the wide and diverse group of peptide antibiotics. Antimicrobial Peptides (AMPs) are found in both vertebrates and invertebrates as well as in plants (Lehrer *et al*., 1993; Nicholas and Mor, 1995; Martin *et al*., 1995). They are small (3.5-4.5 kDa), cationic proteins consisting of 29-42 amino acids which exhibit antimicrobial activity against bacteria, fungi and viruses (Kagan *et al*., 1994; Bals 2000; Tunzi *et al*., 2000). Since, defensins differ in terms of size of their molecules and position of their six cysteine residues they are categorized into three families: α-, β- and θ-defensins (the latter family being the result of cyclization of two α-defensins). Another distinct difference between these defensin families is that the genes that encode them are expressed at different sites.

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β-defensins in vertebrates are synthesized predominantly in the epithelial cells lining various organs, e.g., skin, bronchi, tongue and genitourinary tract (Schonwetter *et al*., 1995; Harder *et al*., 1997; Diamond and Bevins, 1998). Unlike myeloid defensins which are accumulated in vacuoles after phagolysosomal fusion and epithelial defensins are secreted onto the surface of the epithelial tissue (Lehrer *et al*., 1993; Bals, 2010).

Local β-defensin expression patterns which differ with the type of epithelium lining various organs, result in a site-specific composition of antimicrobial peptides most probably due to the specific microflora present on the epithelium surface (Schroder, 1999). Some defensins are released from the epithelium all the time and thus prevent infection others are only released when an inflammatory reaction has been initiated. This indicates that β-defensins play a role as a vital component of the local innate immune system which is activated immediately upon pathogen invasion (Stolzenberg *et al*., 1997; Aono *et al*., 2006; Bagnicka *et al*., 2010). Many of the antimicrobial peptides found in cattle have been classified as β-defensins. The first bovine defensin to be identified was TAP (Tracheal Antimicrobial Peptide) (Diamond *et al*., 1991) which was followed by LAP (Lingual Antimicrobial Peptide) (Schonwetter *et al*., 1995) and EBD (Enteric Beta Defensin) also called DEFB1 (Tarver *et al*., 1998). Moreover, 13 peptides that share homology with β-defensins were isolated from bovine neutrophil granules by Selsted *et al*. (1993).

Bovine β-defensins are encoded by genes consisting of two exons and one intron of about 1.5 kb (Diamond *et al*., 1993; Ganz and Lehrer, 1994; Tarver *et al*., 1998) and are located within one cluster in the synteny group U25 assigned to chromosome BTA27q13-q14 (Gallagher *et al*., 1995). The primary translation product is an inactive precursor (prepropeptide) composed of an N-terminal signal sequence and a C-terminal sequence of a mature peptide which are cleaved by a short pre-sequence (Ganz and Lehrer, 1994). The first exon codes for the signal sequence while the second one codes for the pre-sequence and the mature peptide sequence (Ganz and Lehrer, 1994).

Regardless of the type of defensins, the mechanism of their antimicrobial activity is similar; they quickly damage the cell membrane of the microbe under attack and form pores in it (Risso, 2000). As they penetrate the external and internal membranes of the pathogen, defensins inhibit the synthesis of nucleic acid and proteins and stop the breathing process (Mak, 1994). Defensins are particularly active against gram-positive bacteria and fungi. However, at higher concentrations they are also capable of destroying gram-negative bacteria, mycobacteria, enveloped viruses and some protozoons, e.g., *Giardia lamblia* (Yasin *et al*., 1996; Linde *et al*., 2008). The minimum concentration of hBD-2 (human β-defensins) needed to kill bacteria appears to be much higher *in vitro* than *in vivo*. The reason for this may be that in a live organism it is co-expressed as a group with other AMPs that act synergistically (Lai and Gallo, 2009). Apart from their antibacterial, antifungal and antiparasitic effects, defensins demonstrate immune regulatory properties (Lai and Gallo, 2009). They have a chemotactic effect on monocytes (Yang *et al*., 1999) as well as immature dendritic cells and CD8+memory T lymphocytes (Hancock and Chappel, 1999) which might be crucial for bridging the innate and adaptive immune mechanisms (Yang *et al*., 1999; Garcia *et al*., 2001). Defensins can activate the complement pathway (Yang *et al*., 1999) and modulate the inflammatory process by regulating the production of cytokines (e.g., IL-8) and the expression of adhesion molecules which enable leukocyte migration from the vascular lumen to the site of infection (Chaly *et al*., 2000).

Moreover, defensins enhance lymphocyte proliferation and endotoxin binding and neutralization (Yang *et al*., 2001) inhibit fibrinolysin secretion, stimulate wound healing and promote mast cell degranulation (Ganz, 2002). According to Wetering *et al*. (1997, 2000), defensins inhibit the production of glucocorticosteroids, e.g., cortisol whose secretion is stimulated by ACTH (adrenocorticotropic hormone). They are also known to have antitumor properties (Yang *et al*., 2001; Slemp-Migiel and Wiczkowski, 2006).

The aim of this study was to search for associations between the incidence of clinical mastitis and Combined Defensin Genotypes (CDG) and to investigate the possibility of using defensin gene polymorphisms in marker-assisted selection for immunity to mastitis in dairy cows.

MATERIALS AND METHODS

The study included 1,025 Polish Holstein-Friesian cows kept on a farm located in the North-Western region of Poland. All animals lived under similar environmental conditions. They were kept in one free-stall barn and milked twice a day in a herringbone-type milking parlour. The cows had *ad libitum* access to water from individual automatic drinking vessels and were fed an identical standard TMR (Total Mixed Ration) diet. Additionally, during milking each cow was given specially selected feed concentrate suited to its current physiological condition and milk yield. The cows were of different ages and in different lactations (from 1st to 6th). All clinical cases of

mastitis acuta and mastitis chronica in the herd under study were recorded by a qualified veterinarian employed by the farm. The recorded data included the number of affected udder quarters and the duration of the disease. This study was based on data collected for a total of 3,544 lactations or more specifically inter-calving periods as the cows were examined both during lactation and in the dry-off period (an average of 3.46 lactations per cow).

Genotype identification: DNA was isolated with ZR Genomic DNA Kit™ (ZymoResearch, USA) using Fast-Spin Column technology. The PCR reaction was performed with the following set of primers: F:5'-GCCAGCATGAGGCTCCAT-3' and R:5'-AACAGGT GCCAATCTGT-3'. These primers amplify 1,300-1,650 bp fragments of various *β-DEF* genes (Ryniewicz *et al*., 2003). The PCR mix was composed of 50 ng DNA, 200 μM each dNTP, 20 pmol each primer, 0.75 mM $MgCl_2$, 2 μL 10x Taq1 Buffer, 1 U Taq Polymerase and H_2O to a final volume of 20 μL. The reaction was run for 35 cycles using the following temperature profile: initial denaturation 94°C/300 sec; denaturation 94°C/60 sec, annealing 63.5°C/60 sec, extension 72°C/90 sec; final extension 72°C/60 sec. The amplified fragments were analyzed by the RFLP method (incubation with TaqI enzyme at 65°C for 16 h) then separated electrophoretically on a 2% agarose gel stained with ethidium bromide and visualized under UV light. The reagents used in the laboratory analyses were manufactured by Fermentas (Fermentas International INC, Burlington, Canada) except for primers which were manufactured by Proligo (Proligo France SAS). The PCRs were carried out in thermal cyclers by Whatman Biometra (Whatman Biometra GmbH, Gottingen, Germany). The restriction fragments were visualized using an electrophoresis gel documentation and imaging system by Vilber Lourmat (Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany).

Statistical analysis: The veterinary data was used to analyse both the number of clinical cases and the extent (i.e., the number of infected udder quarters) and duration of infection. The following indicators were applied to cows in each lactation (inter-calving period): -mean number of mastitis acuta cases-MA, mastitis chronica cases-MC, clinical mastitis (acuta and chronica) cases-CM infected quarters-QN, -mean number of days in disease: astitis acuta-MAD, mastitis chronica-MCD, clinical mastitis (acuta and chronica)-CMD and infected quarters/days-QND. To estimate the effect of Combined Defensin Genotypes (CDG) for clinical cases and duration of mastitis, the following Generalized Linear Model (GLM) was used:

$$Y = \mu + CDG + YSC + P + C + e$$

Where:

- y = Value of mastitis indicator (MA, MC, CM, QN, MAD, MCD, CMD and QND)
- μ = Mean value of mastitis indicator (MA, MC, CM, QN, MAD, MCD and SMD) in the population under study
- CDG = Fixed effect of CDG
- YSC = Fixed effect of year/season of calving
- P = Fixed effect of parity
- C = Random effect of cow ~N (θ, $A\sigma^2c$) $\in$A represent an additive polygenic relationships among cows and σ^2c being a component of the additive genetic variance attributed to polygenes
- e = Random residual effect

RESULTS AND DISCUSSION

The primers used in the study enable amplification of fragments of several different β-defensin genes. The PCR yields various products of 1,300-1,650 bp including a 1638 bp fragment of *β1DEF* gene (exons 1 and 2 and intron 1) and fragments of other defensins (Ryniewicz *et al*., 2002). The primers were also 100% complementary to the sequences within *β4DEF* and *β12DEF* genes. Moreover, they made it possible to amplify a fragment of *β5DEF* gene (the reverse primer sequence differs from the gene sequence with one nucleotide only). The amplification products were digested with TaqI enzyme and separated by electrophoresis into individual patterns comprising three pairs of bands named A, B and C. It is impossible to determine if this polymorphism occurs in a single locus or several loci and if a given combined genotype is homo or heterozygous, however there are distinct differences between the individual band patterns.

Thus, the identified genotypes were called by Ryniewicz *et al*. (2002) Combined Defensin Genotypes (CDGs). CDG frequencies were estimated for the whole population under study and for the analyzed set of lactations. As a result, 21 CDGs were identified in the herd (Table 1), the most frequent one being A1A2/B1B2/C1C2 (75.024%). The frequencies of genotypes A1A2/B1B2/C1, A1/B1/C1C2, A2/B1B2/C1C2, A1/B1B2/C1C2, A1A2/B1B2/C2, A1A2/B2/C1C2 and A1A2/B1/C1C2 in the whole herd were >1%. For the purposes of further analyses, all cows with rare genotypes were classified as other (Table 1) because their small number did not allow for accurate estimation of the genotype effect of such genotypes. A statistical analysis showed statistically significant associations between the selected CDGs and all the indicators of susceptibility/immunity to mastitis (Table 2 and 3). I think in methods you need to specify

which test was used to claim significance of CDGs. The least favourable genotype was A1A2/B2/C1C2. Cows with this CDG were most frequently affected by both mastitis acuta and mastitis chronica and had the largest total number of infected udder quarters per cow in a single lactation. Moreover, these animals were found to have the highest number of days in disease (mastitis acuta and mastitis chronica). Genotype A1A2/B1B2/C1 was almost equally unfavourable though it seems that it was associated predominantly with susceptibility to mastitis chronica which manifested itself in a higher number of mastitis chronica cases and accordingly a higher overall incidence of the disease, a higher number of infected udder quarters and a longer duration of infection. However, no statistically significant associations were found between this genotype and the number of mastitis acuta cases (Table 2 and 3).

Conversely, genotype A1/B1/C1C2 was linked to the lowest susceptibility to mastitis. Cows with this genotype developed udder inflammation significantly less frequently and when they did they had the lowest number of infected udder quarters and the condition lasted statistically shorter (Table 2 and 3). Another favourable genotype was A1A2/B1/C1C2 which desired effects included in particular a lower incidence of mastitis chronica and consequently a lower total number of clinical cases and diseased quarters and a shorter total duration of infection. Defensins are multifunctional proteins showing a wide spectrum of antimicrobial activity and therefore, they are considered to be candidate genetic markers for susceptibility/immunity to mastitis (Yang *et al*., 1999; Rainard and Riollet, 2006). Due to their role in protecting the mammary gland against bacterial,

Table 1: Frequency of CDGs in the herd of cows under study

Genotypes	No. of cows in the herd	Frequency (%)	No. of lactations I-VI	Frequency (%)
A1/B1B2/C2*	10	0.976	40	1.129
A1A2/B1B2/C1C2	769	75.024	2647	74.690
A1A2/B1B2/C1	45	4.390	144	4.063
A1/B1/C1C2	18	1.756	57	1.608
A2/B1B2/C1C2	29	2.829	107	3.019
A1/B1/C1*	1	0.098	4	0.113
A1/B1B2/C1C2	33	3.220	117	3.301
A1/B1B2/C1*	7	0.683	29	0.818
A1A2/B1B2/C2	33	3.220	108	3.047
A2/B1B2/C2*	5	0.488	18	0.508
A1A2/B1/C2*	5	0.488	18	0.508
A2/B2/C1*	2	0.195	5	0.141
A1A2/B2/C1C2	19	1.854	78	2.201
A1A2/B1/C1C2	22	2.146	78	2.201
A1A2/B2/C1*	3	0.293	11	0.310
A2/B1B2/C1*	5	0.488	14	0.395
A1A2/B1/C1*	8	0.780	31	0.875
A2/B2/C1C2*	8	0.780	28	0.790
A1/B2/C1C2*	1	0.098	3	0.085
A1A2/B1B1/C1*	1	0.098	4	0.113
A1A2/B2/C2*	1	0.098	3	0.085
Total	1025	100.000	3544	100.000

Due to the low number of cows (N≤10) with the genotypes marked with* these genotypes were considered in further analyses as a single other category

Table 2: Mean number of mastitis clinical cases and mean number of infected udder quarters

		Mastitis acuta cases		Mastitis chronica cases		Clinical mastitis cases		Number of udder quarter	
CDG	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1A2/B1B2/C1C2	2647	0.464	0.952	0.211	0.659	0.675	1.368	0.460^{a}	0.853
A1A2/B1B2/C1	144	0.564	1.040	0.338^{a}	0.878	0.902^{a}	1.637	0.533^{ab}	0.897
A1/B1/C1C2	57	0.278^{a}	0.685	0.148^{b}	0.492	0.426^{aB}	1.092	0.207^{bcDe}	0.466
A2/B1B2/C1C2	107	0.500	0.898	0.216	0.591	0.716	1.172	0.558^{c}	0.910
A1/B1B2/C1C2	117	0.553	1.114	0.193	0.578	0.746	1.368	0.529^{D}	0.958
A1A2/B1B2/C2	108	0.396	0.891	0.151^{c}	0.409	0.547^{c}	1.148	0.411	0.844
A1A2/B2/C1C2	78	0.644^{a}	1.327	0.370^{bcd}	1.061	1.014^{Bcde}	2.111	0.480^{e}	0.841
A1A2/B1/C1C2	78	0.444	0.991	0.111^{ad}	0.358	0.556^{d}	1.209	0.401	0.784
other	208	0.389	0.990	0.192	0.750	0.581^{e}	1.415	0.329	0.689
Total	3544	0.466	0.967	0.213	0.669	0.679	1.384	0.454	0.846

The means marked with capital letters differ at p≥0.01 and those marked with small letters differ at p≥0.05

Table 3: Mean number of days of clinical mastitis

		Days of mastitis acuta		Days of mastitis chronica		Days of clinical mastitis		Number of quarters/days	
CDG	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A1A2/B1B2/C1C2	2647	1.627	3.829	0.671	2.312	2.298	5.160	3.387	8.365
A1A2/B1B2/C1	144	2.023	4.297	1.180^{a}	3.131	3.203^{a}	6.224	5.038^{A}	11.260
A1/B1/C1C2	57	0.833^{ab}	2.126	0.500^{b}	1.851	1.333^{ab}	3.491	1.689^{AB}	5.444
A2/B1B2/C1C2	107	1.784	4.854	0.853	3.046	2.637	7.081	3.628	9.757
A1/B1B2/C1C2	117	2.175^{a}	4.645	0.693	2.074	2.868	5.530	4.206	8.989
A1A2/B1B2/C2	108	1.292	3.341	0.632	1.874	1.925	4.348	2.626c	6.134
A1A2/B2/C1C2	78	2.192^{b}	5.171	1.301^{bc}	3.766	3.493^{bc}	7.816	5.453^{Bcde}	13.751
A1A2/B1/C1C2	78	1.333	2.984	0.389^{ac}	1.449	1.722^{c}	3.962	2.553^{d}	6.204
other	208	1.389	3.723	0.571	2.309	1.960	4.748	2.479^{e}	6.600
Total	3544	1.635	3.891	0.695	2.376	2.330	5.271	3.411	8.502

The means marked with capital letters differ at the p≥0.01 and those marked with small letters differ at the p≥0.05

viral and fungal infections, genes encoding defensins have been suggested as potential markers for the mammary gland immunity (Exner *et al*., 2000). The possibility of using defensin genes as markers related to udder infections in dairy cattle has also been reported by Roosen *et al*. (2004), Ryniewicz *et al*. (2002, 2003) and Bagnicka *et al*. (2007, 2008). The above hypothesis is supported by the discovery that β-defensin is expressed in the cow's udder (Exner *et al*., 2000; Roosen *et al*., 2004) during infection (Goldammer *et al*., 2004; Gunther *et al*., 2009).

Exner *et al*. (2000) also reported the presence of defensin gene transcripts in an infected udder and defensin secretion in mammary epithelial cells. Similarly, Kaiser and Diamond (2000) showed that defensin is produced on the surface of the mammary gland epithelium. Swanson *et al*. (2000) detected infection-induced expression of *LAP* gene in mammary epithelial cells. Roosen *et al*. (2004), on the other hand detected a constitutive expression of *LAP* gene in the udder tissue and other researchers reported such expression in the epithelium of the mammary gland as well as other organs (Schonwetter *et al*., 1995). Furthermore, Roosen *et al*. (2004) discovered that TAP (Tracheal Antimicrobial Peptide) gene was expressed in the udder tissue as well although, it was previously believed to be only expressed in the respiratory epithelium of adult cows (Diamond *et al*., 1993). Roosen *et al*. (2004) also showed the presence of DEFB1 (EBD) mRNA in the udders of infected cows both during lactation and in the dry-off period, suggesting that the expression was induced by pathogens just like Tarver *et al*. (1998) did in the case of intestinal epithelium. DEFB401 and BNBD12 were found to be expressed in response to infection too (Roosen *et al*., 2004).

An interesting pattern of expression in the bovine mammary gland was reported for *BNBD3* and *BNBD9* genes their mRNAs were present during infection but BNBD3 mRNA was also detected in healthy udder quarters while BNBD9 mRNA was only found in quarters displaying symptoms of clinical mastitis (Roosen *et al*., 2004). Noteworthy is the fact that the mRNAs of genes encoding β-defensins which are normally present in neutrophil granules were also found in the mammary gland. It is rather unlikely that the mRNAs originated from neutrophils since mature defensin peptides are known to be formed in myeloid cells and their synthesis is already complete when mature neutrophils are released from the bone marrow into the peripheral circulation (Yount *et al*.,1999). Yount *et al*. (1999) who discovered BNBD4, -12 and -13 mRNAs in the larynx, lungs, spleen and intestines were of the opinion that neutrophils might also be stimulated to express β-defensin genes in some tissues. Another explanation might be that β-defensins are expressed in macrophages migrating to the site of infection. For example, Ryan *et al*. (1988) detected β-defensin mRNA in alveolar macrophages in the lungs of infected cows. Mastitis-induced expression of β-defensins is initiated by LPS (Lipopolysaccharides) conserved pathogenic endotoxins which stimulate adequate TRLs (Toll-like Receptors), leading to the activation of the NF-κB (Nuclear Factorsss kappa B) transcription factor signalling pathway (Diamond *et al*., 2000a; Goldammer *et al*., 2004; Compton *et al*., 2009).

Schroder (1999) demonstrated that the local pattern of expression characteristic of defensins is determined by the specific composition and density of microflora present on the surface of epithelia. Using the *in situ* hybridization method, Swanson *et al*. (2000) detected defensin expression on the surface of the mammary gland tissue and found a positive association between Somatic Cell Count (SCC) in milk and defensin expression which indicates that defensin plays a major role in the immune response in the udder.

The researchers concluded that the secretion of defensin into milk protects the mammary gland from bacterial invasion and as a result might influence milk quantity and composition. Defensins are present not only in the mammary gland but also in leukocyte granules (Diamond *et al*., 2000b; Frye *et al*., 2000) and macrophages (Zhang *et al*., 1998) which constitute a large portion of the somatic cell population in milk and phagocytize microorganisms (Fehlbaum *et al*., 2000). Moreover, studies by Ryniewicz *et al*. (2002, 2003) showed statistically significant associations between CDGs and SCC in milk, daily milk yield and percentage fat and protein content in milk in Black and White cows (Ryniewicz *et al*., 2002) as well as in Black and White cows with a large share of Holstein-Friesian genes (Ryniewicz *et al*., 2003).

Wojdak-Maksymiec *et al*. (2006) reported that in a herd of Jersey cows the lowest SCC (transformed to a logarithmic scale) was characteristic of animals with A1/B1/C1C2 genotype while cows with A2/B1B2/C1C2 genotype had the highest SCC in milk. The researchers also found statistically significant associations between CDGs and daily milk yield: cows with genotypes A1A2/B1B2/C2 and A2/B1B2/C1 had the highest milk yield while those with genotypes A2/B1/C1C2 and A1A2/B1/C1 had the lowest milk yield. The percentage content of milk fat and protein demonstrated an inverse relationship with milk yield: it was lower in cows which yielded most milk and higher in cows with the lowest milk

yield (Wojdak-Maksymiec *et al*., 2006). The association between bovine β4-defensin gene polymorphism and SCC in milk was also reported by Krzyzewski *et al.* (2008) and Bagnicka *et al*. (2007, 2008).

While most studies carried out so far have focused on searching for associations between defensin gene polymorphism and SCC in milk, no researchers have investigated associations between defensin genes and the incidence of clinical mastitis diagnosed on the basis of veterinary examinations. Although, SCC is quite strongly correlated with the actual cases of mastitis (Mrode *et al*., 1998; Rupp and Boichard 1999), it must be borne in mind that it is merely an indirect diagnostic criterion and a high SCC does not always have to be a sign of infection. Therefore, before defensin genes can be used in marker-assisted selection for improved innate immunity to mastitis it is necessary to investigate thoroughly the associations between the genes in question and the actual incidence of udder inflammation. This task was undertaken by Kumarverma *et al.* (2009). Unfortunately, probably due to the small number of cows included in their study they were unable to confirm any statistically significant associations between CDGs and clinical mastitis.

CONCLUSION

Only the results of the present study make it possible to verify if whether defensin gene polymorphisms in particular CDGs can be used in breeding programmes aimed at enhanced innate immunity to mammary gland infections in dairy cattle. A matter that needs to be looked into in the future areis the biological mechanisms underlying the different effects of some genotypes on chronic and acute infections. Presumably, the differences result from the regulatory functions of defensins in the immune system.

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