TRANSLATIONAL RESEARCH IN VETERINARY SCIENCE
Vol 3, No 2, 2020
DOI: http://dx.doi.org/10.12775/TRVS.2020.006
ISSN 2657-4489 (online)

Association of bovine *CXCL8* polymorphism with clinical mastitis and fertility trait in Polish HF cattle

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Abstract

Background: The aim of this study was to investigate the relationship between *CXCL8* (*CXCL8*c.105A>G and *CXCL8*c.210C>T) SNP polymorphism and the clinical mastitis and production trait in Polish HF cattle.

Methods: The trait-associated *CXCL8* SNPs polymorphism study was carried out in the Polish HF bulls (n = 151) by PCR-RFLP methods as described in the previous issue of this journal. The phenotypic records (assessment year 2017) of clinical mastitis, functional and fertility trait were collected from the Research Institute of Animal Production, Balice, Poland (http://www.izookrakow.pl).

Results: Overall, the trait-associated study revealed no relationship between the *CXCL8*c.210C>T SNP polymorphism and the breeding value of selected clinical mastitis and fertility trait of breeding bulls. However, TT genotypes showed low levels of significance of differences for the breeding values of somatic cells count (p = 0.27) and stillborn calving of heifers (p=0.27). Similarly, the trait-associated study showed no correlation between the *CXCL8*c.105A>G polymorphism and the breeding value of selected clinical mastitis and fertility trait of breeding bulls. However, AA genotypes showed low levels of significance of differences for the somatic cell count (p = 0.12), ease calving of heifers (p = 0.14), the difficulty calving of heifers (p = 0.17), live-born calves of heifers (p = 0.21), and AG genotypes showed the low levels of significance of differences for the stillborn calving in heifers (p = 0.21).

Conclusions: Study concludes that trait-associated studies of *CXCL8* polymorphism did not identify highly significant effects on clinical mastitis and fertility trait in the investigated breeding bulls of Polish HF cattle.

Keywords: trait-associated; breeding values; *interleukin-8*; *CXCL8*; somatic cell count; clinical mastitis; fertility trait; Polish HF.

Introduction

Bovine mastitis is with clinical and subclinical forms remains the most common and costly disease of dairy cattle worldwide resulting in significant economic losses because of its negative impacts on animal welfare, productive, and reproductive performances poor milk quality increased workload early culling and high treatment costs [**1–4**]. In cattle, identification of candidate genes that underlie complex traits such as susceptibility to clinical mastitis are the major goal of many genetic and biomedical studies, which provides mechanistic insights into host resistance in addition to improving the diagnosis and treatment of the disease. In a recent studies identification of candidate genes for clinical mastitis in

Norwegian cattle were reported using the genome-wide association study (GWAS) approaches and transcriptome analysis [**5–6**]. In other trait-associated studies, candidate genes for clinical mastitis viz., bovine *Toll-like receptor (TLR)* and pro-inflammatory cytokine genes families including the chemokine *CXCL8* were reported as potentially biomarkers associated the clinical mastitis in cattle [**7–9**]. Herein this paper, we investigate the relationship between the chemokine *CXCL8* (*CXCL8*c.105A>G and *CXCL8*c.210C>T) SNP polymorphism and the breeding values records of clinical mastitis and fertility trait in Polish HF bulls.

Materials and methods

Animals: The study investigated 151 randomly selected Polish HF bulls with known breeding values from 57 proven sires. The official results of the assessment of breeding values of investigated bulls were obtained from the Research Institute of Animal Production, Balice website (http://www.izookrakow.pl). The data was taken from the year 2017 assessment.

Methodology procedures: The PCR-RFLP of *CXCL8* SNPs polymorphism was carried out in the Polish HF bulls, according to the methodological procedure as described in the previous issue of this journal [**10**]. Based on the assessment of breeding values of bulls in the calendar year of 2017, the phenotypic records of clinical mastitis, functional and fertility trait (Milk trait, longevity, somatic cell content, Dead calves of bull daughters, live calves of bull daughters, non-repeatability index of estrus for heifers, non-repeatability index of estrus for cows, inter-pregnancy interval, easy births of bull's daughters, difficult births of bull's daughters, abortion in bull's daughter, postpartum after birth, udder position, udder width, setting of teats, teats length, suspended front replacement, and suspended rear replacement) were collected from the Research Institute of Animal Production, Balice, Poland (http://www.izookrakow.pl).

Statistical procedures: The study results were compiled taking into account the frequency of genotypes and alleles. The state of genetic equilibrium was assessed using the mathematical formula of the Hardy

– Weinberg law. The chi-square test was used to verify the consistency of the expected and observed frequency of genotypes. Using statistical package STATISTICA 12.0, the influence of *CXCL8*c.105A>G and *CXCL8*c.210C>T SNP polymorphism on the breeding value of bulls was estimated by the two-factor and three-factor analysis of variance, according to the formula as below:

$$Yijkl = \mu + G_{1i} + G_{2j} + S_k + e_{ijkl}$$

where:
Y_{ijkl} – investigated trait,
μ – average mean value of the phenotypic trait,
G_{1i} (I = 1,2,3) – effect of *CXCL8*c.210C>T SNP genotypes, CC, CT, TT,
G_{2j} (j = 1,2,3) – effect of *CXCL8*c.105A>G genotypes, AA, AG, GG,
S_k (n = 1.....57) – permanent effect of K^{th} bull,
e_{ijkl} – random error.

The assumptions for the analysis of variance (ANOVA) for the normality of the distribution were checked by the Shapiro-Wilk test, while the homogeneity of variance were checked by the Leven test. The significance of differences between the arithmetic means was determined on the basis of Duncan's test.

Results and discussion

In this study, we investigated the bovine *CXCL8* candidate gene for clinical mastitis in Polish HF cattle. The trait-associated study was performed using the breeding values of Polish HF bulls. Obtained results from trait-associated study showed no significant association between the *CXCL8*c.210C>T polymorphism and the breeding values indices of selected clinical mastitis and fertility trait in Polish HF bulls (**Table 1**). However, the genetic association with lowest significance of differences were observed for the easy calving (p = 0.09) with highest among homozygous

Table 1. Genetic association between the *CXCL8*c.210C>T polymorphism and the breeding values indices of selected clinical mastitis and fertility trait in Polish HF bulls

Phenotypic Trait	Overall		Genotypes						P value
			CC		CT		TT		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
	n = 151		n = 40		n = 76		n = 35		
milk trait	99.4	0.51	99.2	0.87	99.7	0.79	99.3	0.92	0.89
longevity	91.8	0.64	91.2	1.34	91.8	0.88	92.9	1.24	0.63
somatic cell count	95.3	0.97	92.7	2.46	96.1	1.19	96.5	1.73	**0.27***
stillborn calves in heifers	4.74	0.17	4.55	0.29	5.02	0.22	4.41	0.42	**0.27***
live calves in heifers	95.2	0.17	95.5	0.29	95.0	0.22	95.6	0.42	**0.27***
non-repeatability index of estrus for heifers	101.8	0.79	103.1	1.79	101.9	1.06	100.7	1.57	0.57
non-repeatability index of estrus for cows	98.3	0.82	97.5	1.41	98.9	1.29	99.1	1.47	0.77
inter-calving interval	98.8	0.81	98.5	1.63	98.9	1.12	98.7	1.69	0.97
easy calving in heifers	63.3	1.29	65.2	2.80	60.5	1.44	66.9	3.17	**0.09***
difficult calving in heifers	2.99	0.17	2.60	0.27	3.00	0.21	3.33	0.53	0.35
abortion in heifers	1.09	0.07	0.96	0.12	1.12	0.09	1.16	0.17	0.53
postpartum after birth	97.7	0.81	96.9	1.74	97.8	1.10	97.91	1.59	0.87
udder position	96.8	0.73	96.3	1.56	96.8	1.02	96.9	1.35	0.94
udder width	99.6	0.55	100.0	1.24	100.0	0.76	98.4	0.89	0.46
setting of teats	98.0	0.68	97.1	1.47	98.0	0.87	99.4	1.53	0.49
teats length	99.0	0.85	98.1	1.60	99.4	1.22	99.6	1.67	0.77
suspended front replacement	96.9	0.75	95.7	1.38	97.1	1.09	97.4	1.53	0.67
suspended rear replacement	98.6	0.48	98.6	0.91	98.7	0.69	98.4	0.98	0.97

*The lowest significance of differences is marked in bold letters.

TT (66.9) and lowest among heterozygotes CT (60.5) bulls. Moreover, similar trends were also observed for the number of somatic cells counts ($p = 0.27$), stillborn calves ($p = 0.27$), and live calves ($p = 0.27$) with highest significance of differences among TT genotype and the lowest among CC genotypes.

Similarly, no significant association was observed in the trait-associated study between the *CXCL8*c.105A>G polymorphism and the breeding values indices of selected clinical mastitis and fertility trait in Polish HF bulls (**Table 2**). However, the genetic association with lowest significance of differences were observed for the longevity ($p = 0.29$), somatic cell count ($p = 0.12$), and difficult calving ($p = 0.17$) with highest among homozygous AA and lowest among homozygous GG bulls, whereas, in case of live calves ($p = 0.21$) and easy calving ($p = 0.14$) with highest among homozygous AA and lowest among heterozygotes AG bulls. Lastly, in case of stillborn calves ($p = 0.21$), the genetic association with lowest significance of differences were observed, with highest among heterozygotes AG and lowest among homozygous AA bulls.

Several studies were reported the clinical mastitis and fertility related trait-associated studies to investigate the candidate genes for mastitis resistance such as: *mitogen activated protein 4 kinase 4 (MAP4K4)* gene in Chinse Holstein cattle [**11**], *toll-like receptor (TLR2)* and *caspase recruitment domain 15 (CARD15)* genes in Canadian Holstein cattle [**12**], *toll-like receptors (TRL4)* gene in Holstein bulls [**13**], *cytokine-activated Janus kinase 2 (JAK2)* and *signal transducer and activator of transcription 5B (STAT5B)* genes in Chinese cattle [**14**], and *osteopontin (SPP1)* gene in Canadian Holstein cattle [**15**]. Using PCR RFLP technique, significant associations between the -79T>G SNP and the 3'UTR +2463 C>T SNP and susceptibility to clinical mastitis were identified in the investigated in HF cattle [**16**]. Study further reported that clinical mastitis rates for genotypes -79 TT and +2463 TT were much lower in comparison to the homozygous genotypes (-79 GG and +2463 CC) and significantly lower than their respective heterozygous genotypes. In our study, we found the clinical mastitis and fertility trait indices (somatic cells counts, stillborn calves, live calves) for *CXCL8*c.210C>T SNP maker were higher for the homozygous

Table 2. Genetic association between the *CXCL8*c.105A>G polymorphism and the breeding values of selected clinical mastitis and fertility trait in Polish HF bulls

Phenotypic Trait	Overall		Genotypes						P value
			AA		AG		GG		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
	n = 151		n = 40		n = 76		n = 35		
milk trait	99.4	0.50	98.8	0.92	99.7	0.80	99.6	0.85	0.76
longevity	91.8	0.64	93.2	1.20	92.0	0.86	90.4	1.41	**0.29***
somatic cell count	95.3	0.98	96.7	1.68	96.4	1.18	91.9	2.53	**0.12***
stillborn calves in heifers	4.73	0.17	4.37	0.40	5.05	0.23	4.56	0.30	**0.21***
live calves in heifers	95.3	0.17	95.6	0.40	95.0	0.23	95.4	0.30	**0.21***
non-repeatability index of estrus for heifers	101.8	0.78	100.6	1.50	101.9	1.07	103.3	1.79	0.49
non-repeatability index of estrus for cows	98.3	0.81	98.9	1.54	98.9	1.31	97.6	1.30	0.79
inter-calving interval	98.8	0.81	99.6	1.78	98.9	1.14	97.6	1.48	0.68
easy calving in heifers	63.3	1.29	66.5	3.03	60.7	1.44	65.0	2.90	**0.14***
difficult calving in heifers	2.99	0.18	3.43	0.50	3.00	0.21	2.49	0.27	**0.17***
abortion in heifers	1.09	0.07	1.18	0.16	1.08	0.08	1.00	0.13	0.65
postpartum after birth	97.7	0.81	98.7	1.68	97.8	1.12	96.2	1.63	0.54
udder position	96.8	0.73	96.9	1.28	96.9	1.03	96.0	1.61	0.86
udder width	99.6	0.55	9.78	0.79	100	0.78	99.6	1.31	0.67
setting of teats	98.0	0.68	98.6	1.47	98.1	0.88	97.6	1.52	0.86
teats length	99.0	0.85	99.6	1.84	99.5	1.24	97.8	1.44	0.68
suspended front replacement	96.9	0.75	97.5	1.46	97.2	1.10	95.3	1.40	0.50
suspended rear replacement	98.6	0.48	98.8	0.92	98.8	0.70	98.2	0.94	0.88

*The lowest significance of differences is marked in bold letters.

TT and lower for the heterozygotes CT breeding bulls. Whereas, for the *CXCL8*c.105A>G marker, the clinical mastitis and fertility trait indices (longevity, somatic cells count, difficult calving, live calves and easy calving) were higher for the homozygous AA and lower for the heterozygotes GG and AG breeding bulls.

Conclusions: Study concludes that trait-associated studies of *CXCL8* polymorphism did not identify highly significant effects on clinical mastitis and fertility trait in the investigated breeding bulls of Polish HF cattle.

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