Acta agriculturae Slovenica, 92(december 2008)2, 185–187.

http://aas.bf.uni-lj.si

Agris category codes: L73 COBISS Code 1.03

POLYMORPHISM ANALYSIS OF THE PROMOTER OF COW LACTOFERRIN GENE WITH PCR-RFLP AND ITS CORRELATION WITH SUBCLINICAL MASTITIS

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Received July 23, 2008, accepted December 06, 2008.
Delo je prispelo 23. julija 2008, sprejeto 06. decembra 2008.

ABSTRACT

CMT was used to detect the incidence of mastitis. One hundred twenty cows were selected and assigned into 2 groups, 60 animals in each group: control group (healthy cows), experimental group (cows with subclinical mastitis) and the relationship between cow′s subclinical mastitis and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the promoter of cow lactoferrin gene was explored. The results showed that polymorphism existed in the promoter of bovine lactoferrin gene, which suggested that this polymorphism could be associated with mastitis susceptibility.

Key words: cattle / dairy cows / diseases / subclinical mastitis / molecular genetics / lactoferrin gene / PCR-RFLP

PCR-RFLP ANALIZA POLIMORFIZMOV V PROMOTORJU LAKTOFERINSKEGA GENA PRI GOVEDU IN POVEZAVA S SUBKLINIČNIM MASTITISOM

IZVLEČEK

Mastitis smo ugotavljali na osnovi kliničnih znakov. Sto dvajset krav smo razdelili v dve skupini, 60 živali je bilo v kontrolni skupini (zdrave živali), drugih 60 pa v poskusni skupini (živali s subkliničnim mastitisom) in ugotavljali povezavo med subkliničnim mastitisom in polimorfizmom restrikcijskih fragmentov (PCR-RFLP) promotorske regije gena za laktoferin. Rezultati kažejo, da bi bil polimorfizem v promotorski regiji govejega laktoferinskega gena lahko povezan z dovzetnostjo za mastitis.

Ključne besede: govedo / krave / molznice / bolezni / subklinični mastitis / molekularna genetika / geni / laktoferin / PCR-RFLP

INTRODUCTION

The milk lactoferrin (Lf) is synthesized mainly by breast epithelial cells and neutrophils and secreted as the non-heme iron-binding protein, belonging to the transferrin family (Plaffl *et al*., 2003). In some studies authors reported that in infected cows, the milk and serum concentration of Lf will change (Hirvonen *et al*.,1999; Barkema, 1998). In cows with mastitis Lf may compete with bacteria for iron ions, so the RFLP analysis of genetic polymorphism in Lf gene, and relationship with udder infections may have theoretical and practical significance.

Through this study Lf gene promoter region was analyzed using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP), and possible association with mastitis susceptibility was tested.

MATERIALS AND METHODS

Sample Collection

Animals from the Xinjiang Shihezi dairy cattle farm were divided into a healthy group (control group) and group with subclinical mastitis (test), 60 in each group. From each animal was collected 10 mL blood, mixed with anticoagulant and frozen at –20 °C.

Test Method

The PCR was performed in a final volume of 25μL containing 40 ng of template DNA, 20 pmole of each primer (5'-CACATTACAAGCAGGATCTTTTGCTG-3' and 5'-CTGGCCAATGAGCCCTATATGTGT-3'), PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1 mM $MgCl_2$, 0.25 mM of dNTPs, and 0.5 U of *Taq* DNA polymerase. This solution was initially denatured at 94 °C for 4 min. followed by 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 45 s), and elongation (72 °C for 45 s) and a final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1.5% agarose gels in order to check the quality and specificity of DNA fragment amplification. To examine the nucleotide sequence variation at the *Lf* locus, the *Hin*f I restriction enzyme was chosen. The resulting DNA fragments were separated on 6% PAGE gels, using *pBR322* as molecular marker. Gels were photographed under UV light with a Gel Doc 1000 system (BioRad) after ethidium bromide staining and the relative migration of the DNA bands was estimated.

RESULTS

Amplification of the Lf gene fragment revealed a 1143 bp long product after electrophoresis of PCR product (Fig. 1). After restriction enzyme digestion with *Hin*fI the two alleles were characterized by two and one restriction fragment, respectively. The heterozygotes showed three bands (Fig. 2, Table 1)

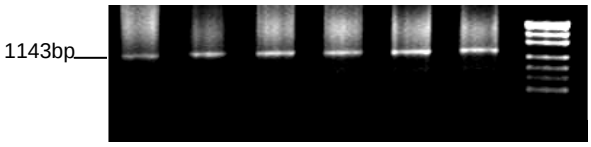

Figure 1. Gel electrophoresis of PCR products of the lactoferrin gene promoter fragment.
Slika 1. Gelska elektroforeza PCR produkta promotorja laktoferinskega gena.

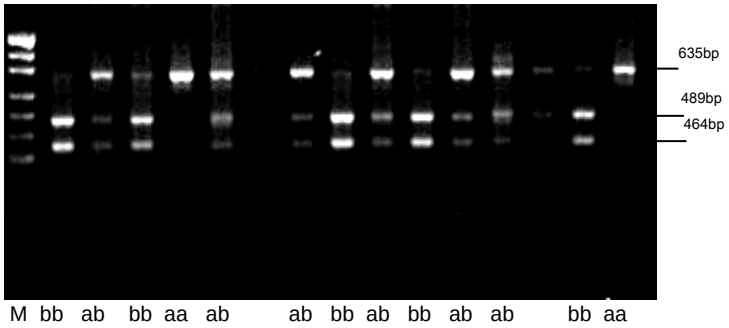

Figure 2. PCR-RLFP patterns of lactoferrin gene promoter PCR products digested with *Hin*fI.
Slika 2. PCR-RLFP vzorci fragmenta laktoferinskega promotorja po restrikciji s *Hin*fI.

Table 1. Genotype frequency and gene frequency in the promoter of lactoferrin gene
Preglednica 1. Frekvence genotipov in alelov v promotorju laktoferinskega gena

Groups	Sample Sizes	Genotype frequency			Gene frequency	
		A / A	A / B	B / B	A	B
Control group	60	0.5	0.4	0.1	0.78	**0.22**
Experimental group	60	0.15**	0.23	0.62**	0.17**	0.83**

** $P < 0.01$

Hardy-Weinberg Equilibrium Test

The results of the exact Fisher test for the significance of Hardy-Weinberg probabilities are shown in Table 1. The frequencies for homozygotes AA and BB, as well as allele frequencies in the experimental group show significant departure from H-W equilibrium.

DISCUSSION

This study revealed variation in RFLP banding pattern of the Lf gene promoter PCR fragment, which might be associated with the level of the Lf gene expression. Our results support the hypothesis that this mutation might be associated with mastitis susceptibility. Further research is needed to investigate the possible effect of the described mutation on Lf mRNA and protein level in milk.

REFERENCES

Barkema, H.W. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts[J]. J Dairy Sci, 81(1998)2, 411–419.

Hirvonen, J./ Eklund, K./ Teppo, A.M. *et al.* Acute phase response in dairy cows with experimentally induced mastitis[J]. Acta Vet Scand, 40(1999), 35–46.

Plaffl, M.W./ Wittmann, S.L./ Meyer, H.H.D./ Bruckmaier, R.M. Gene expression of immunologically important factors in blood cells,milk cells and mammary tissue of cows [J]. J Dairy Sci, 86(2003)3, 538–545.