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Relationship between beta lactoglobulin and subclinical mastitis in Valle del Belice sheep breed

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ABSTRACT: The objective of the following research was to determine the effect of LGB genotypes on subclinical mastitis in Valle del Belice dairy sheep. Ewes were classified as affected or not by subclinical mastitis within a lactation based on i) a positive culture in one of the test-days and ii) more than 750,000 somatic cells. Generalized linear mixed models were fitted to assess the significance of LGB genotypes on MTB and MTC. The LGB genotypes significantly affected MTB ($p=0.0387$), and showed a tendency on MTC ($p=0.1104$). Least square means showed that in the analysis for MTB, individuals with genotypes BB and AB had a higher frequency of subclinical mastitis. Moreover, the least square differences showed that the incidence was significantly higher in BB ewes than in AA ewes ($p<0.001$), and a tendency of a higher incidence among AB ewes than in AA ewes ($p=0.0630$). In conclusion, the results of this work show that LGB genotype BB seems to be less favourable in terms of mastitis resistance in Valle del Belice sheep breed.

Key words: Subclinical mastitis, Molecular marker, β-lactoglobulin, Sheep.

INTRODUCTION – Mastitis is the most common and costly pathology affecting dairy animals. The economical aspects are related not only to loss of milk production but also to treatments cost. Clinical mastitis produces evident changes in the mammary gland and in the milk yield and quality (Barrillet et al. 2001; Gonzalo et al. 2002). These changes make the identification of affected animals relatively easy.

Subclinical mastitis, on the other hand, is difficult to be detected, and the final diagnosis is based on the isolation of pathogens from milk culturing. Somatic cell count (SCC) is a powerful tool to identify animals with subclinical mastitis (Clements *et al.* 2003; Moroni *et al.* 2005). The udder inflammation produced an increase on leukocyte cells through infiltration from the bloodstream, evidenced by an increment in SCC in milk. However, mastitis is not the only cause of SCC variation. Other factors such as age, stage of lactation, and level of milk production should be considered when SCC is analyzed (for a review, Bergonier *et al.*, 2003; Moroni *et al.* 2007).

The current methods to control mastitis rely on prophylactic measures and antibiotic treatments. The identification of a genetic marker that allows the inclusion of mastitis resistance in selection programs would help to reduce the economic impact due to this disease as well as the use of antibiotics. However, the selection of a candidate gene is a difficult task because mastitis is a complex disease influenced by many genes and environmental factors (e.g. milking system and stocking density). β-lactoglobulin (LGB) is a member of the lipocalin protein family and plays a role in the retinol transport and in the regulation of the immune response (for a review see Flower, 1996). LGB polymorphism has previously been associated with SCC in dairy cows (Kriventsov *et al.*, 1975; Walawski *et al.*, 1997; Luhar *et al.*, 2006). This gene has three genetic variants: A, B and C in sheep (Erhardt, 1989), being the allele A and B the most prevalent. Kriventsov *et al.* (1975) have reported a lower total milk microbiota in animals producing LGB B than in animals expressing LGB A. Walawski *et al.* (1997) found an association between genotype

AA for the LGB gene and a high level of SCC in cows. On the other hand, Luhar *et al.* (2006) found that allele B is associated with mastitis. There are no studies reported in sheep. Therefore, the aim of this research was to determine the effect of LGB genotypes on SCC and on milk bacteriological analysis in Valle del Belice sheep.

MATERIAL AND METHODS – *Sample collection.* A total of 776 lactations, collected between July 2004 and June 2006, from 433 Valle del Belice ewes were used. Milk samples for bacteriological culture were aseptically taken and kept refrigerated until analysis. Milk samples were collected with preservation and frozen until processing for Isoelectrofocusing (IEF). Blood samples were collected with EDTA. *SCC.* values were measured automatically by Fossomatic 5000 (Foss Spa). *Bacteriological analyses.* The following bacteria were identified: Corynebacterium spp., Pasteurella spp., Pseudomonas spp., coagulase negative staphylococci, Staphylococcus aureus, Staphylococcus intermedius, Streptococcus canis, Streptococcus dysgalactiae, Streptococcus uberis, and Streptococcus agalactiae. Milk culture was considered positive when one or more colonies were isolated from the milk sample. *IEF.* Milk samples were phenotyped by IEF to infer LGB genotypes (Erhardt. 1989). Among other proteins, IEF allows to recognize lactoglobulin proteins A and B in milk. *DNA extraction and PCR protocols.* DNA was extracted from EDTA-blood samples (GFX genimsc blood purification kit, Amersham Biosciences). Amplifications were performed in a Thermocycler Geneamp PCR system 9700 (Applied Biosystems) primers sequences, PCR reaction and enzymatic restriction (RsaI, Fermentas) were performed as described by Feligini *et al.* (1998). *Statistical Analysis.* The ewes were classified, within lactation, as affected or not based on i) having a positive culture in one of the test-days (MTB, mastitis based on bacteriological analysis) and ii) having more than 750,000 somatic cells (MTC, mastitis based on somatic cell counts). These two traits were analyzed with GLIMMIX – SAS (2004). Generalized linear mixed models with a logit link function and binomial distributions were fitted to assess the significance of LGB genotype effect on MTB and MTC; these models included LGB genotype (3 levels) flock (4), year (3) and season (2) of lambing, number of offspring per lambing (2), and lactation order (2) as fixed effects, and the ewe as random effect to model repeated records.

RESULTS AND CONCLUSIONS – LGB genotypes were inferred by IEF and confirmed by PCR-RFLP in a subset of the sample. Frequencies of LGB genotypes against MTB and MTC are shown in Table 1.

Table 1. Frequencies of LGB genotypes on MTB and MTC ewes.

	MTB					MTC				
	0		1			0		1		
LGB	count	freq.	count	freq.	LGB	count	freq.	count	freq.
AAa	23	0.46	27	0.54	AAa	21	0.42	29	0.58
ABa,b	114	0.31	254	0.69	ABa	147	0.40	221	0.60
BBb	86	0.24	272	0.76	BBa	107	0.30	251	0.70

Statistically significant differences (α = 0.05) in LGB genotypes are indicated by different superscript letters.

The LGB genotypes significantly affected MTB (p=0.0387), and showed a tendency on MTC (p=0.1104). Least square means showed that, in the analysis for MTB, ewes with genotypes BB and AB presented higher mastitis incidence than those with genotype AA. Furthermore, the least square differences showed that the incidence was significantly higher in BB ewes than in AA ewes (p=0.0353) while there was just a tendency of a higher incidence among those AB ewes than in AA ewes.

In conclusion, the results of this work indicate that LGB genotype BB are less favorable in terms of mastitis resistance. These results are in agreement with those reported in cows (Kriventsov *et al.*, 1975; Walawski *et al.*, 1997; Luhar *et al.*, 2006) where different LGB genotypes were associated with resistance or susceptibility to mastitis. However, the allele associated with mastitis susceptibility reported in cattle is different among the authors.

Our results might indicate that LGB or a closely linked gene could be responsible for the resistance to mastitis. LGB could be use as molecular marker for mastitis resistance, however, it is important to underline that different environments may influence the susceptibility to pathologies. Especially since mastitis is a very complex disease and the environment plays a decisive role in the development of the mammary gland inflammation, further studies are needed to determine whether the genetic polymorphism is associated with resistance or susceptibility to diseases in any particular breed.

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