Assessment of genetic variation for pathogen-specific mastitis resistance in Valle del Belice dairy sheep

M. Tolone[1], C. Larrondo[2], S. Newman[3], A.M. Sutera[1], R. Di Gerlando[1], M.T. Sardina[1], S. Mastrangelo[1], J.M. Yáñez[4], B. Portolano[1]

[1] Università degli Studi di Palermo, Dipartimento Scienze Agrarie, Alimentari e Forestali, Viale delle Scienze. 90128 Palermo, Italy
marco.tolone@unipa.it (Corresponding Author)
[2] Faculty of Veterinary Sciences, Austral University of Chile, Valdivia, Chile
[3]Genus PLC, Hendersonville, TN, USA
[4] Faculty of Veterinary and Animal Sciences, University of Chile, Santiago, Chile

Summary

Mastitis resistance is a complex and multifactorial trait, and its expression depends on both genetic and environmental factors, including infection pressure. The objective of this research was to determine the genetic basis of mastitis resistance to specific pathogens using a repeatability threshold probit animal model. The most prevalent isolated pathogens were coagulase-negative staphylococci (CNS); 39% of records and 77% of the animals infected at least one time in the whole period of study. There was significant genetic variation only for *Streptococci* (STR). In addition, there was a positive genetic correlation between STR and all pathogens together (ALL) (0.36 ± 0.22), and CNS and ALL (0.92 ± 0.04). The results of our study support the presence of significant genetic variation for mastitis caused by *Streptococci* and suggest the importance of discriminating among different pathogens causing mastitis due to the fact that they most likely influence different genetic traits. Low heritabilities for pathogen specific-mastitis resistance may be considered when including bacteriological status as a measure of mastitis presence to implement breeding strategies for improving udder health in dairy ewes.

Keywords: mastitis, ewes, pathogens, resistance

Introduction

Mastitis is one of the most common diseases affecting dairy sheep. Mastitis leads to major economic losses, mainly due to discarded milk, reduced milk production and quality, alteration of cheese-making properties, early culling, and increased health care costs. Mastitis resistance is a complex and multifactorial trait, and its expression depends on both genetic and environmental factors. In dairy sheep the most important agents involved in clinical mastitis are the bacterial infections, and the most frequently isolated pathogens are coagulase-negative staphylococci (CNS), that are present on and around the udder skin with a different pathogenicity causing clinical and subclinical mastitis (Pengov, 2001). There are few studies concerning genetic variation of mastitis in sheep according to bacteriological status (Tolone *et al.*, 2013). Tolone *et al.* (2013) reported genetic variation accounting for resistance to mastitis in Valle del Belice dairy sheep. These authors defined mastitis as a binary trait distinguishing between ewes with at least one case of mastitis (1) and ewes without (0) in a defined period of lactation, and analyzed it using a linear model approach. This definition excluded alternative definitions, for example multiple cases of mastitis within lactation, and ignored the

etiology of intra-mammary infections. The purpose of this study was to determine the genetic bases of pathogen-specific resistance to mastitis in Valle del Belice dairy sheep using a repeatability threshold probit animal model.

Material and methods

Data

Data were collected between 2006 and 2011 in five Valle del Belice flocks, with a total of 2,350 ewes and 5,856 animals in the pedigree. Observations for this study included 1,795 primiparous, 1285 secondiparous, and 2,225 multiparous dairy ewes. All ewes were milked twice daily, and records for milk yield (MY), bacteriological status (infected or not infected), and SCC were collected at approximately monthly intervals, following an A4 recording scheme which is defined by the International Committee for Animal Recording (ICAR, 2014). Sample collection, animal management and cares were in agreement with the Directive 2010/63/EU. The observed bacteriological colonies were identified as: *Escherichia coli* (ESCCL), *Staphylococcus aureus* (STHAU), *Streptococcus dysgalactiae* (STPDG), *Streptococcus uberis* (STPUB), *Streptococcus agalactiae* (STPAG) and *Bacillus spp.* (BACIL), *Corynebacterium spp.* (CORLT), *Pasteurella spp.* (PASCL), *Pseudomonas spp.* (PSELT), coagulase negative staphylococci (CNS) and *Streptococcus spp.* (STR). Ewes were considered infected if more than five colony forming units (CFU) per 10 µl of milk of one species of bacteria were isolated, while they were considered healthy if the bacteriological test did not show a positive result. Data from ewes were collected more than one time during the same lactation. Thus, the repeatability of records is across and within lactations. The response variable used in the model corresponds to the binary disease status, coded as 0 or 1 to represent uninfected or infected individuals, respectively.

Trait definition and statistical model

Phenotypic observations of infection status were defined as a repeated binary trait: sheep with infected (1) and uninfected (0) udder status within each lactation for each particular pathogen. SCC was normalized into somatic cell score (SCS) according to Ali & Shook (1980). The binary trait was analyzed using the following repeatability threshold probit animal model:

$$\boldsymbol{Pr(Y_{ijklm}) = \Phi(\mu + OP_i + MY_j + FYS_k + PE_l + A_m)}$$

Where y_{ijklm} is the observation for the specific pathogen causing mastitis; Φ is the normal cumulative density function; µ is the fixed effect of the overall mean; OP_i is the order of parity fitted as fixed effect (with 5 classes); MY_j is the milk production yield fitted as covariate; FYS_k is the flock-year-season random effect (51 classes); PE_l is the random permanent environmental effect of the individual *m* across lactations (2350 levels with records); and A_m is the random animal effect (5856 levels in the pedigree). The implicit residual variance on the underlying scale is 1 for the probit model. Parameters of the univariate threshold models were estimated using ASREML version 3.0 (Gilmour *et al*., 2009).

Heritabilities

Heritabilities for resistance to different pathogens were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{FYS}^2 + \sigma_{PE}^2 + \sigma_e^2}$$

Where σ_a^2 is the animal additive genetic variance, σ_{FYS}^2 is the variance associated with flock-year-season, σ_{PE}^2 is the variance due to permanent environment and σ_e^2 is residual variance. For all traits, the animal effect was assumed ~ N(0, Aσ_a^2), where A is the additive genetic relationship matrix among all animals included in the pedigree. Similarly, FYS and PE effects were assumed ~ N(0, Iσ_{FYS}^2) and ~ N(0, Iσ_{PE}^2), where I is an identity matrix with order equal to number of FYS and PE classes (51 and 2350), respectively.

Results and Discussion

Absolute (AF) and relative (RF) frequency distribution according to infection status (infected or uninfected) observations within all the records data set, are shown in Table 1. The most prevalent isolated pathogens were CNS with 7,951 (39%) observations, followed by STHAU (940; 5%) and STR (541; 3%). Considering all pathogens (ALL), 8,019 (38%) milk samples were infected. Table 2 shows AF and RF frequency distributions according to udder status (infected or uninfected) of animals per pathogen. Similarly, the most prevalent isolated pathogens affecting ewes were CNS (1,811; 77%), followed by STHAU (513; 21.83%) and STR (217; 9.23%) when only animals were analyzed. Table 3 shows components of variance due to FYS, PE, additive genetic effect, phenotypic effect and heritabilities for resistance to CNS, STR, and ALL. Due to the low frequency of isolation of ESCCL, STHAU, STPDG, STPUB, STPAG, BACIL, CORLT, PASCL, and PSELT in the total records and isolation of pathogens per animals, problems associated with convergence were found. In contrast, there was statistically significant genetic variation for CNS, STR, and ALL pathogens (Table 3). The heritability obtained for STR (0.09) was significant different from zero, whereas for CNS (0.02) and ALL (0.02) were not. All of the estimated phenotypic and genetic correlations were significantly different from zero (Table 4). The phenotypic correlation between ALL and STR was low, however, the genetic correlation was moderately high indicating that there was a direct relationship between these traits in genetic terms. On the other hand, both phenotypic and genetic correlations between ALL and CNS were high, indicating that there was a strong positive relationship, both phenotypic and genetic, between these traits. These results suggest that resistance to CNS is similar to the resistance when it is measured as ALL. In addition, the phenotypic correlation between STR and CNS was negative and the genetic correlation between these traits was low, indicating that selection for improved STR will not have an impact on CNS resistance. Mean SCS for uninfected and mean SCS of whole data set were similar to the values reported in Valle del Belice (Riggio *et al.*, 2010) and Churra dairy sheep breeds (Ariznabarreta *et al.*, 2002). Our study confirms that CNS is the most prevalent etiological group of bacteria in the infected dairy ewes. Moreover, a high percentage (77%) of animals was found infected at least one or more times in the period of study, showing the importance of this group of bacteria in this population. Besides, due to the high prevalence of CNS during the ewe's lactations, subclinical cases could persist, significantly increase SCC and consequently cause clinical mastitis. Considering the opportunistic nature of CNS, with adequate hygiene practices, correct milking routine and periodic revision of milking equipment, intramammary infections by CNS could be reduced. STHAU was the second one more frequently isolated bacteria in our study (5%) followed by STR (3%). For STHAU, ewes infected at least one time or more in the period of study were 22% (513). These findings

are different respect to what reported by Riggio *et al.* (2013) and similar to other studies ranging from 2 to 5.5% (Pengov, 2001; Ariznabarreta *et al.*, 2002). Infection due to STHAU is related with subclinical to acute clinical mastitis with different clinical symptoms according to the virulence of the strains and in severe cases lead towards culling of the affected sheep. In sheep, a heritability estimate of 0.09 for infection status assessed by bacteriological analyses was reported by Riggio *et al.* (2013) and Tolone *et al.* (2013) in the Valle del Belice breed using a threshold animal model assuming a probit link function. Gonzalo *et al.* (2003) estimated genetic parameters of SCC in Churra sheep considering the type of mammary pathogen using a multitrait repeatability animal model. They reported that the effect related to the type of pathogen accounted for 32.5% of the total variance in SCC, a value similar to that obtained for the residual effect (34.9%), indicating a high relative importance of the type of pathogen in the decomposition of the variance for SCC. These results showed the importance of differentiating between the types of mammary bacteria assessed by bacteriological analyses in genetic mastitis studies. Variances due to permanent environment and FYS effects were high and were important factors to explain the phenotypic variance resistance against CNS, STR and ALL. CNS group of bacteria are related with inadequate management and hygiene practices, which could be different among the flocks, through the year and among them. Therefore, due to poor flock management and inadequate milking hygiene could increase the probability of occurrence of mastitis, and flocks may act as reservoirs of some CNS species. Heritabilities for pathogen-specific mastitis were in agreement with results of De Haas (2003) in dairy cattle ranging from 0.02 to 0.10. However, this study only included heritabilities of pathogens involved in clinical mastitis cases and were estimated through threshold and linear models. For genetic correlations, the one estimated between CNS and ALL (0.92) was positive and very high suggesting that both are the same traits. This could be explained for the high frequency of isolation of CNS in the records (77%). Thus, a high percentage of ALL group is explained by CNS pathogens. Furthermore, due to the fact that phenotypic variation for CNS and ALL is determined primarily by an environmental component both type of traits (CNS and ALL) could be controlled more effectively by applying correct management measures instead of selective breeding on this population. In the Valle del Belice breed, where the current selection is mainly practiced on a “within farm” approach and based on own performance of ewes, it is unlikely that selection for mastitis resistance is successful, independent of the use of infection status or SCS.

Conclusion

The results of our study support the presence of significant genetic variation for resistance to one specific pathogen causing mastitis (i.e. *Streptococci*). The high genetic correlation between ALL and CNS indicate that both are almost the same trait. The opportunistic nature of CNS and the high environmental influence of CNS resistance suggest that improvement of flock management and adequate milking hygiene could reduce significantly the incidence of mastitis caused by this pathogen in Valle del Belice dairy sheep.

Table 1. Absolute (AF) and relative (RF) frequency distribution according to udder status of observations (n = 20,519).

Pathogen	Status[1]	AF	RF
CNS	0	12,568	0.61
	1	7,951	0.39
STHAU	0	19,579	0.95
	1	940	0.05
STR	0	19,978	0.97
	1	541	0.03
ALL	0	13,246	0.62
	1	8,019	0.38

[1] Udder status as binary record: 0 = not infected, 1 = infected
CNS coagulase-negative *staphylococci*, *STHAU Staphylococcus aureus*, *STR streptococci*, *ALL* observed bacteriological colonies as described in M&M section

Table 2. Absolute (AF) and relative (RF) frequency distribution according to udder status of animals (n = 2,350) per pathogen.

Pathogen	Status[1]	AF	RF
CNS	0	539	0.23
	1	1,811	0.77
STHAU	0	1,837	0.78
	1	513	0.22
STR	0	2,133	0.91
	1	217	0.09
ALL	0	612	0.26
	1	1,738	0.74

[1] Udder status as binary record: 0 = not infected, 1 = infected
CNS coagulase-negative *staphylococci*, *STHAU Staphylococcus aureus*, *STR streptococci*, *ALL* observed bacteriological colonies as described in M&M section

Table 3. Estimates of components of variance and their standard errors for infectious status.

Resistance Trait Infection Status	σ^2_{FYS}	σ^2_{PE}	σ^2_a	σ^2_P	h^2
ALL	0.79 ± 0.17	0.40 ± 0.03	0.04 ± 0.02	2.23 ± 0.17	0.02 ± 0.01
CNS	0.18 ± 0.04	0.39 ± 0.03	0.03 ± 0.02	1.60 ± 0.05	0.02 ± 0.01
STR	0.09 ± 0.03	0.39 ± 0.07	0.15 ± 0.07	1.62 ± 0.05	0.09 ± 0.04

σ^2_{FYS} Flock-Year-Season effect, σ^2_{PE} permanent environment effect, σ^2_a) additive genetic effect, σ^2_P phenotypic effect, h^2 heritabilities
CNS coagulase-negative *staphylococci*, *STR streptococci*, *ALL* observed bacteriological colonies as described in M&M section

Table 4. Phenotypic (above diagonal) and genetic (below diagonal) correlations and standard errors for resistance to mastitis.

Infection status	STR	CNS	ALL
STR	-	-0.08 ± 0.02	0.17 ± 0.01
CNS	0.24 ± 0.25	-	0.87 ± 0.01
ALL	0.36 ± 0.22	0.92 ± 0.04	-

CNS coagulase-negative *staphylococci*, *STR streptococci*, *ALL* observed bacteriological colonies as described in M&M section

List of References

Ali A, Shook G. 1980. An optimum transformation for somatic cell count in milk. J Dairy Sci. 63:487-490.

Ariznabarreta A, Gonzalo C, San Primitivo F.2002. Microbiological quality and somatic cell count of ewe milk with special reference to staphylococci. J Dairy Sci. 85:1370–1375.

De Haas Y. 2003. Somatic cell count patterns Improvement of udder health by genetics and management. PhD Thesis, Wageningen University.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R. 2009. ASReml User Guide Release 3.0 VSN International Ltd, Hemel Hempstead, HP1 1ES, UK www.vsni.co.uk.

Gonzalo C, Ariznabarreta A, Othmane M, Carriedo J, De La Fuente L, San Primitivo F.2003. Genetic parameters of somatic cell count in dairy sheep considering the type of mammary pathogen effect. J Anim Breed Genet. 120:282-287.

ICAR (International Committee for Animal Recording). 2014. International agreement of recording practices. Available online: http://www.icar.org/wp-content/uploads/2015/11/Guidelines.

Pengov A. 2001. The role of coagulase-negative Staphylococcus spp and associated somatic cell counts in the ovine mammary gland. J Dairy Sci. 84:572-574.

Riggio V, Pesce LL, Morreale S, Portolano B. 2013.Receiver-operating characteristic curves for somatic cell scores and California mastitis test in Valle del Belice dairy sheep. Vet J. 196:528-532.

Riggio V, Portolano B, Bovenhuis H, Bishop S. 2010. Genetic parameters for somatic cell score according to udder infection status in Valle del Belice dairy sheep and impact of imperfect diagnosis of infection. Gen Sel Evol. 42:30.

Tolone M, Riggio V, Portolano B. 2013. Estimation of genetic and phenotypic parameters for bacteriological status of the udder, somatic cell score, and milk yield in dairy sheep using a threshold animal model. Livest Sci. 151:134-139