ASSOCIATION BETWEEN *PrP* GENOTYPE AND MILK PERFORMANCE IN COMISANA SHEEP BREED

F. Pinelli[1], M. Scimonelli[1], P.A. Oltenacu[2], F. Vitale[3], M. Vitale[3], A. D'Amico[1], S. Caracappa[3], A. Carlucci[4]

[1] Istituto Sperimentale Zootecnico per la Sicilia - ISZS, Palermo, Italy
[2] Department of Animal Science, Cornell University, Ithaca, NY, USA
[3] Istituto Zooprofilattico Sperimentale della Sicilia - IZSS, Palermo, Italy
[4] Associazione Provinciale Allevatori di Matera, Italy

INTRODUCTION

Scrapie is an infectious disease of sheep that affects the central nervous system and is always fatal. Upon necropsy, infected animals will have holes or vacuoles in the tissue of the brain. The disease is classified as a transmissible spongiform encephalopathy (TSE). Relatively recent evidence that ties a new variant of Creutzfeldt-Jakob Disease in humans with eating meat from BSE-infected cattle has resulted in increased emphasis on scrapie eradication.

Sheep prion protein (*PrP*) is a protein of 256 amino acids in length. PrP^{Sc} and PrP^{C} have the same amino acids sequence but different molecular structures. The prion infectious agent theory contends that PrP^{Sc} infects a normal sheep and serves as a vector converting PrP^{C} to the infective shape of PrP^{Sc}. Amino acid changes in at least three locations (amino acids number 136, 154, and 171) have been shown to confer increased or decreased susceptibility to scrapie (Goldmann *et al*. 1994; Ikeda *et al*. 1995; Hunter *et al*. 1997b; Hunter *et al*. 1997a; Thorgeirsdottir *et al*. 1999; Tranulis *et al*. 1999). The haplotypes *ARH*, *AHQ* and *ARR* are associated with increasing resistance to scrapie, while the haplotypes *ARQ* and *VRQ* are associated with increasing susceptibility. It was also reported that in breeds in which *VRQ* allele is not present or is very rare, animals homozygote for *ARQ* allele are very susceptible to scrapie infection (Hunter *et al*. 1997).

European Union requires member states to implement breeding programs for resistance to scrapie in each sheep breed (Decision 2003/100/EC). The objective is to increase the frequency of *ARR* allele and reduce the frequencies of *ARQ* and *VRQ* alleles. Four classes were defined to describe the degree of resistance to scrapie: class 1 and 2 for sheep that are genetically most resistant and resistant, and class 3 and 4 for sheep that are genetically susceptible and highly susceptible (defined in table 1). In Italy such a program was started in 2005 for each native breed within the flocks officially registered. Based on the breeding program guidelines, all breeding rams in participating flocks must be genotyped for *PrP* gene, rams carrying *VRQ* allele must be slaughtered and only class 1 and class 2 rams can be used for breeding.

While anti-scrapie selection programs have already started, it is important to have good knowledge regarding *PrP* allele and genotype frequencies for each breed. We also need to evaluate the association between *PrP* genotypes and economically import traits to insure that selecting for increased resistance to scrapie does not have undesirable consequences for each breed. The association between prion protein genotype and performance traits was evaluated by De Vries *et al*. (2004) for meat breeds of sheep, by De Vries *et al*. (2005) for East Friesian Milk Sheep and by Barrillet *et al*. (2002) for Lacaune and Manech breeds. Comparable studies are not available for Comisana breed.

Since 1998, ISZS has implemented a nucleus breeding program to improve milk production in Comisana sheep, involving a group of commercial farms in Sicily (Pinelli *et al*. 2002). In 2005, selection for scrapie resistance was introduced in the breeding program. The objectives of this study were: first, to evaluate a possible association between *PrP* genotypes and milk production traits used in selection; and second, to evaluate the possible effect of eliminating susceptible alleles *VRQ* and *ARQ* from the nucleus on milk yield.

MATERIALS AND METHODS

Data comes from two flocks that represent the multiplication and ram center components of the breeding program. Blood samples were collected on 838 ewes and 232 rams. Detection of *PrP* genotype was performed at IZSS. DNA was extracted from EDTA-treated blood using a DNA isolation kit for mammalian blood (Nucleomag 96 blood) from Macherey-Nagel and allelic discriminations were performed by reverse hybridization method using the Kit Ovine *PrP* Gene Test, from Nuclear Laser Medicine, following manufactures' instructions.

Milk production records for 838 ewes with known *PrP* genotype were used to analyze the association between the *PrP* genotype and milk yield. These records included 5337 test-day milk records taken according to the A4 method. The test day data were analyzed with a mixed linear model that included flock (1,2), parity (1,2 and 3+), days in milk interval (26 DIM intervals of 10 days) and *PrP* genotype (1,…,13) as fixed effects, and ewes and residuals as random effects. The covariance between test day yields within ewe was modeled with compound symmetry structure. The same model was used to evaluate the effect of susceptible alleles on milk yield. To evaluate the effect of gene substitution on milk yield for *VRQ* and *ARQ* alleles, the *PrP* genotypes were defined with respect to *VRQ* allele as 2, 1 and 0 (*VRQ*/*VRQ*, *VRQ*/*XXX* and *XXX*/*XXX*, respectively) and similarly with respect to *ARQ* allele. The analyses were performed using SAS mixed model procedure with repeated measures.

RESULTS AND DISCUSSION

The frequencies of *PrP* genotypes for 1070 animals are shown in Table 1. In our data, the frequencies of "scrapie resistance" alleles *ARH*, *AHQ* and *ARR* were 0.0285, 0.0275 and 0.3935 and the frequencies of "scrapie susceptible" alleles *ARQ* and *VRQ* were 0.5108 and 0.0397, respectively. The frequencies for the four classes of resistance/susceptibility to scrapie were 165, 480, 342 and 83 animals, corresponding to 15.42%, 44.86%, 31.96% and 7.76%, respectively. Interesting to note that in Comisana breed two alleles, *ARR* and *ARQ*, have very high frequencies while all other alleles are at very low frequencies. The *VRQ* allele has a very low frequency and its elimination from the population should be easy to accomplish. Therefore, for Comisana breed, the breeding program should focus on increasing the frequency of *ARR* allele and reducing the frequency of *ARQ* allele.

Table 1. Frequencies of the *PrP* genotypes in Comisana dairy sheep

PrP genotype	Scrapie class resistance	Frequency	Percent
ARR/ARR	1	165	15.42
ARR/ARH	2	19	1.78
ARR/AHQ	2	19	1.78
ARQ/ARR	2	442	41.31
ARQ/ARQ	3	275	25.70
ARQ/AHQ	3	28	2.62
AHQ/AHQ	3	2	0.19
ARQ/ARH	3	17	1.59
ARH/ARH	3	11	1.03
AHQ/ARQ	3	9	0.84
VRQ/VRQ	4	2	0.19
VRQ/ARQ	4	47	4.39
VRQ/ARH	4	1	0.09
VRQ/AHQ	4	1	0.09
VRQ/ARR	4	32	2.99

To evaluate the effect of *PrP* genotype on milk yield per day, the genotypes with only one observation per class were not included. The least squares means and standard errors describing the effect of *PrP* genotypes on milk yield per day (g) are shown in Table 2.

Table 2. Least Squares Means and their Standard Errors for the effect of *PrP* genotype on milk yield per day (g)

PrP genotype	Estimate	St. Error
ARR/ARR	875.91	56.04
ARR/ARH	816.33	53.50
ARR/AHQ	808.05	15.02
ARQ/ARR	877.57	18.11
ARQ/ARQ	872.02	55.30
ARQ/AHQ	894.23	219.99
AHQ/AHQ	1023.10	64.06
ARQ/ARH	988.02	54.66
ARH/ARH	909.87	75.87
AHQ/ARQ	692.26	129.33
VRQ/VRQ	678.07	36.89
VRQ/ARQ	833.65	34.66
VRQ/ARR	932.01	56.04

Flock, DIM, parity and DIM(parity) were significant (P<.0001) while the effect of *PrP* genotypes was not significant. These results are in agreement with De Vries *et al*. (2005) and Barillet *et al*. (2002), both reporting no significant association between *PrP* genotypes and milk performance. It is interesting to note that the Least Squares Means for the three most frequent genotypes in the population, *ARR*/*ARR*, *ARR*/*ARQ* and *ARQ*/*ARQ*, are essentially equal (876, 877 and 872 grams of milk per day, respectively) indicating that changes in the frequencies of *ARR* and *ARQ* alleles may not negatively affect milk yield.

The same results were obtained for the *VRQ* and *ARQ* substitution effects. The regression coefficients measuring the phenotypic effect of gene substitution for *VRQ* and *ARQ* allele were negative (-0.553 and -0.065, respectively) but not significantly different from zero.

CONCLUSIONS

The data analyzed in this study show no evidence of an association between *PrP* genotypes and milk performance in Comisana sheep breed.

The results of this study indicate that a program designed to increase resistance to scrapie in Comisana breed should not affect milk yield and, therefore, can be incorporated into the breeding program for milk improvement. Nevertheless, programs should be implemented to monitor inbreeding rate in the population and possible negative effects on fitness traits and other important breed characteristics to insure that intensive selection aimed at moving the breed toward the status of 'scrapie resistant' is safe with respect to the genetic fitness of the breed as well as its economic relevance.

REFERENCES

Barillet, F., Andreoletti, O., Palhie`re, I., Aguerre, X., Arranz, J. M., Minery, S., Soulas, C., Belloc, J. P., Briois, M., Fre´geat, G., Teinturier, P., Amigues, Y., Astruc, J. M., Boscher, M. Y. and Schelcher, F. (2002). *Proc*. 7th *WCGALP* **31** : 683–686.

De Vries, N., Hamman, N., Drogemuller, C., Reinecke, S., Lupping, W. and Distl, O. (2004) *Veterinary Record* **155** : 140-143.

De Vries, F., Borchers, N., Hamman, N., Drogemuller, Ganter, M. and Distl, O. (2005) *J. Dairy Sci.* **88** : 392-398.

Goldmann, W., Hunter, N., Smith, G., Foster, J. and Hope, J. (1994) *J. General. Viology* **75** : 989-995.

Hunter, N., Goldmann, W., Foster, J. D., Cairns, D. and Smith, G. (1997a). *Veterinary Record* **141** : 137-140.

Hunter, N., Moore, L., Hosie, B. D., Dingwall, W. S. and Greig, A. (1997b). *Veterinary Record* **141** : 59-63.

Ikeda, T., Horiuchi, M., Ishiguro, N., Muramatsu, Y., Kai-Uwe, G. D. and Shinagawa, M. (1995) *J. General Virology* **76** : 2577-2581.

Pinelli, F., Oltenacu, P.A., Iannolino, G., Scimonelli, M., Romeo, G., D'Amico, A. Carvalheira, J., Carlucci, A. and Vella, A. (2002). *Proc. 7th WCGALP* Com. N° 01- 38.

Thorgeirsdottir, S., Sigurdarson, S., Thorisson, H. M., Georgsson, G. and Palsdottir A. (1999) *J. General Virology* **80** : 2527-2534.

Tranulis, M. A., Osland, A., Bratberg, B. and Ulvund, M. J. (1999) J. General Virology **80** : 1073-1077.