PRION GENE OCTAREPEAT VARIABILITY IN THE ITALIAN CATTLE BREEDS

P. Leone[1], B. Castiglioni[1], T. Sechi[2], P. Cassini[3] and A. Stella[3]

[1]C.N.R., Istituto Difesa e Valorizzazione del Germoplasma Animale, 20090 Segrate, Italy
[2]Istituto Zootecnico e Caseario, Olmedo (SS), Italy
[3] CERSA, 20090 Segrate, Italy

INTRODUCTION

The Bovine Spongiform Encephalopathies (BSE) is a neuro-degenerative disease characterised by the accumulation of an abnormal protease-resistant isoform of the prion protein in brain. The bovine prion gene extends over 20 kb, and the mRNA, consisting of three exons, is 4244 bp long. In the coding region of the gene, only one polymorphism has been identified to date, in a octapeptide repeat region, that can contain either 5, 6, or 7 copies of a motif of 8/9 amino acids (Pro, Gln, Gly, [Gly], Gly, Gly, Trp, Gly, Gln). To date, polymorphism of this octarepeat region is not correlated to incidence of BSE, although the high proportion of genotype 6:6 was demonstrated among the affected animals (Goldman *et al.*, 1991). This polymorphism has been analysed in several cattle breeds and the results are summarised in Table 1.

Table 1. known allele frequencies in bibliography

Breed	B (1)	HF (2)	HE (2)	HF (3)	S (4)	HF (4)	B (4)	G (4)	I (4)	S (4)	BU (4)
(n)	72	10	46	183	10	9	5	2	22	12	3
Allele											
5	.15	.03	.01	.06	.03	0	.4	0	0	.38	.17
6	.70	.97	.99	.94	.97	1	.6	1	1	.62	.83
7	.15	0	0	0	0	0	0	0	0	0	0

B=Brown Swiss, HF=Holstein Friesian, HE=Hereford, S=Simmental, G=Grey, I=Istrian, S=Slavonian Syrmian, BU=Busa, (1)=Schlapfer *et al.,*1999; (2) =Brown *et al.*,1993; (3)= Neibergs *et al*., 1994; (4) =Premzl *et al.*, 2000.

In Italy, testing for the presence of BSE became obligatory in January 2001 and to date (February 2002) more than 522,000 analyses have been performed, among which 56 affected animals were found. Fifty-one of those cases were attributed to local breeds, 35 to the Frisona Italiana, 10 to the Bruna Alpina and 6 to the Pezzata Rossa breed.
In this study we examine a sample representative of the Italian cattle population to determine the allelic frequencies and distribution of the prion gene.

MATERIAL AND METHODS

Animals. 755 non-related animals of 14 Italian cattle breeds (Bruna Alpina/BA, Chianina/CH, Frisona Italiana/FI, Grigio Alpina/GA, Marchigiana/MA, Maremmana/MM, Modicana/MD, Pezzata Rossa Valdostana/PR, Piemontese/PI, Podolica/PO, Reggiana/RG, Rendena/RE, Romagnola/RO, and Sarda/SA) were analysed.

Analytical methods. DNA was isolated from peripheral lymphocytes using standard isolation procedures.

Experiment 1. The PCR primers were described by Premzl *et al*. (2000). The PCR reaction mixture, in a final volume of 20 µl, contained 100-200 ng DNA, 1x Taq Buffer 10x (Sigma), 0,2 mM dNTPs (Pharmacia), 0,1 µM of each primer and 1 U Taq-Polymerase (Sigma). The reaction was performed in 35 cycles (94°C, 1 min, 65°C, 1 min, 72 °C, 1 min). The PCR products were analysed by electrophoresis in 3% ethidium bromide-stained agarose gel.

Experiment 2. Two samples representative of each polymorphism were sequenced to verify the exact sequence of the octapeptide repeat (Goldman *et al.*, 1991; Schlapfer *et al.,*1999). Primers and reaction conditions described by Mackenzie *et al*. (199), were used to perform sequences in a final volume of 20 µl; the reaction mixture contained 100 ng of DNA, 1x Taq buffer 10x (Finnzymes), 10 µl dNTPs solution (Pharmacia) e 1 U Taq DNA Polymerase (Finnzymes). The reactions were performed in 25 cycles (96°C, 10 sec, 50°C, 5 sec, 60°C, 4 min) and the sequences were obtained by electrophoresis on ABI 310 apparatus.

Statistical analysis. Genotypic frequencies were calculated and the populations was verified to be in Hardy-Weinberg equilibrium (HWE).

RESULTS AND DISCUSSION

Three alleles were identified, characterised by 5, 6, or 7 repetitions of the octapeptide sequence resulting, in DNA fragments of 349 bp, 373 bp, and 397 bp respectively. All the six genotypes (5:5, 5:6, 5:7, 6:6, 6:7, 7:7) were found (Figure 1).

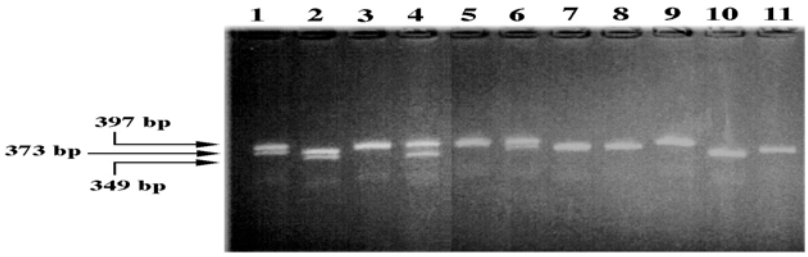

Figure 1. PrP gene amplification products on a 3% agarose gel

Figure 1 shows the DNA amplification products on an agarose gel. Lanes 1, and 6 represent genotype 6:7; lane 2 represents genotype 5:6; lanes 3, 5, and 9 represent genotype 7:7; lane 4 represent genotype 5:7; lanes 7, 8, and 11 represent genotype 6:6; and lastly lane 10 shows genotype 5:5.

The obtained sequences(data not shown) confirm the presence of the different series of octarepeats, characterising the three alleles.
The animals presenting the allele with six copies of the octarepeats were the most frequent in all the breeds with exception of the Bruna Alpina, in which the animals with the allele with seven copies prevailed (Table 2).

Table 2. frequency of alleles in the Italian cattle breeds

Breed (n)	BA (96)	CH (42)	FI (96)	GA (62)	MA (40)	MM (51)	MD (16)	PI (51)	PO (40)	PR (74)	RE (48)	RG (57)	RO (35)	SA (47)
Allele														
5	.21	0	.02	.02	0	0	0	.06	0	.03	.23	.03	0	.05
6	.38	1	.98	.98	1	1	1	.94	1	.97	.77	.97	1	.95
7	.41	0	0	0	0	0	0	0	0	0	0	0	0	0

As expected, the genotype 6:6 was the most common in all of breeds (1 for CH, MA, MM, MD, PO, and RO; 0,97 for RV and GA; 0,95 for RG and FI; 0,92 for PI; 0,64 for RE; 0,95 for SA), except the Bruna Alpina (0,14). The genotype 5:5 was found only in the breeds RV (0.01), PI (0.02) BA (0.07), and RE (0.11). The allele with seven repetitions was identified only in the Bruna Alpina breed. This allele was previously found in the Brown Swiss breed (Schlapfer *et al*.,1999), and provide a proof of a common origin of the two breeds. This allele was the most frequent form in the BA and animals were both homozygous 7:7 (0.15), and heterozygous 5:7 (0.19), and 6:7 (0.34). (Table 3).

Table 3. Comparison between observed frequency and expected genotypic frequencies

Breed	BA		FI		GA		PI		RE		RG		PR		SA	
Allele	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp	obs	exp
5 5	.073	.044	0	.001	0	0	.020	.004	.104	.053	0	.001	.015	.001	0	.003
5 6	.104	.160	.052	.058	.032	.040	.059	.112	.250	.354	.053	.058	.027	.058	.106	.095
5 7	.188	.172	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6 6	.146	.144	.948	.941	.968	.960	.921	.884	.646	.593	.947	.941	.948	.941	*.894*	.902
6 7	.344	.312	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7 7	.145	.168	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P	.110		.696		.746		.024		.043		.772		.000		*.666*	

Table 3 shows the genotypic frequencies for the breeds in which polymorphism was found, and P-values of tests for HWE. Frequencies within the PI, RE and PR differed from HWE ($P<0.05$), suggesting some sort of non-random mating. In general, departures from HWE in these breeds were due to higher than expected frequencies of the 5:5 homozygous genotype.

CONCLUSION

A comparison of the frequencies found in this study with those previously published confirms low variability in the octapeptide region with regard to the number of alleles and their distribution. However, analysis of polymorphism at the PrP locus allows to group breeds according to geographical distribution within the country. Breeds without variability at the locus are originally from central-southern Italy. Although in Italy, to date, it was not possible to confirm the absence of correlations of octarepeat variability and the pathologic phenotype. The high proportion of genotype 6:6 is of particular concern because genotype 6:6 is consistently present of among the affected animals (Goldmann et al., 1991).

REFERENCES

Brown, D. R., Zhang, H. M., DeNise, S. K. and Ax, R. L.(1993) *Anim. Biotech.* **4** (1) : 47-51.

Goldmann, W., Hunter, N., Martin, T., Dawson and M., Hope, J. (1991*) J. Gen. Virol.* **72** : 201-204.

McKenzie, D. J., Cowan, C. M., Marsh, R. F. and Aiken, J. M. (1992*) Anim. Biotech.* **3** (2) : 309-315.

Neibergs, H.L., Ryan, A. M., Womack, J. E., Spooner, R. L. and Williams, J. L. (1994) *Anim. Genet*. **25** : 313-317.

Premzl, M., Bozic, P. and Gamulin, V. (2000) *Anim. Genet*. **31** : 408-409.

Schlapfer, J., Saitbekova, N., Gaillard and C., Dolf, G. (1999) *Anim. Genet.* **30** : 386-387.