Genetic diversity of *NRAMP1* 3'-UTR microsatellite in cattle breeds reared in Sardinia

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ABSTRACT - The aim of this study was to compare the allele frequency of 3'-UTR *NRAMP1* (Natural Resistance-Associated Macrophage Protein) microsatellite between local and specialized dairy cattle breeds reared in Sardinia, Italy. Blood samples were collected and DNA was extracted from 97 Sarda, 55 Italian Brown and 36 Italian Friesian cattle and analysed by means of PCR and PCR-SSCP. On the whole, three alleles were found, GT_{13}, GT_{14}, and GT_{15}. GT_{13} showed the highest frequency in all the breeds: 0.874 in the Sarda, 0.973 in the Italian Brown and 1 in the Italian Friesian. For the Sarda, both GT_{14} and GT_{15} showed a frequency of 0.063, while for the Italian Brown 0.018 and 0.009, respectively. Homozygous GT_{13}/GT_{13} was the unique genotype for the Italian Friesian and the most representative for the Italian Brown (0.964) and Sarda (0.823). The other genotypes for the Sarda were: GT_{14}/GT_{14} (0.042), GT_{13}/GT_{14} (0.010), GT_{13}/GT_{15} (0.094) and GT_{14}/GT_{15} (0.031); as regards the Italian Brown, both GT_{14}/GT_{14} and GT_{13}/GT_{15} showed a genotypic frequency of 0.018. The observed heterozygosity was lower than the expected value both for the Sarda and the Italian Brown. Sarda showed a higher genetic variability than Italian Brown and Italian Friesian.

Key words: Natural resistance, Cattle, Sarda breed, Heterozygosity.

Introduction - Infectious diseases are a relevant cause of economic losses in the field of animal husbandry. Furthermore, in the last decades, the massive use of antibiotic has led to drug resistance and residue in food of animal origin. These problems stimulated both the drafting of new laws in the European Union to limit the use of antibiotic and coccidiostatic drugs (Regulation EC No 1831/2003), and the study of new methods other than antibiotics to fight against infectious diseases. A modern approach is based on the knowledge of immunological mechanism in order to obtain the improvement of the natural resistance of animals (Womack, 1988). Natural resistance to disease is described as the innate capacity of an animal to resist disease when it is exposed to pathogens, without prior exposure to immunization (Hutt, 1958). The mechanism of natural resistance is strictly linked to the efficiency of the immunological response and under genetic control (Barger, 1989). Some studies identified a locus on murine chromosome 1, which was named *Bcg*/*Ity*/*Lsh* because of its importance in the resistance against Mycobacterium Bovis (bacillus Biliè-Calmette-Gueren) (Bcg), Salmonella Typhimurium (Ity) and Leishmania Donovani (Lsh) (Crocker *et al*., 1984). The gene, later renamed *NRAMP1*, the acronym of Natural Resistance-Associated Macrophage Protein (Vidal *et al*., 1993), codes for a transmenbrane protein of transporter family which regulates the activity of macrophages (Blackwell, 1996). A GT_n microsatellite is located at the 3' untranslated region (3'-UTR) of the bovine *NRAMP1* gene and its polymorphism is associated with natural resistance against brucellosis (Adams and Templeton, 1998). In cattle, the allele GT_{13} is associated with resistance, while GT_{14} and GT_{15} with susceptibility to brucellosis (Barthel *et al*., 2000, 2001), but the real implication of this polymorphism

in the mechanism of inhibiting bacterial growth has not been clarified yet. In a study by Paixao *et al*. (2006), genotypic frequencies are quite different between Holstein and Zebu cattle (100% and 31% of GT_{13}/GT_{13} homozygotes, respectively). The aim of this study was to compare the allele frequency of 3'-UTR *NRAMP1* microsatellite between local and specialized dairy cattle breeds reared in the island of Sardinia, Italy.

Material and methods - A total of 187 cattle (96 Sarda, 55 Italian Brown, and 36 Italian Friesian) from 18 farms were selected. All cattle were registered in their respective herd book and descended from different sires and dams. DNA was extracted from individual blood samples and the microsatellite at 3'-UTR *NRAMP1* amplified by means of PCR (Feng *et al*., 1996). PCR products were later denatured and genotyped by means of PCR-SSCP (Single Strand Conformation Polymorphism) method as previously described by Barthel *et al*. (2000). Electrophoresis was carried out in a 8% acrylamide gel in a D-CODE System for SSCP (BIO RAD) and the reaction parameters were the following: 25W, 1000V and 150mA for 2h, constant temperature 12°C. SSCP fragments were visualized after Sybr-Gold staining, purified using ChargeSwitch® PCR Clean-UP Kit (Invitrogen) and sequenced by means of the ABI PRISM 3730 DNA Analyzer (Applied Biosystems). Nucleotide sequences were evaluated using the software Bioedit (Sequence Alignment Editor, Hall, 1999). Data were later analysed by means of two different software programs. GenePop (Raimond and Rousset, 1995) was used to calculate allelic and genotypic frequencies and Hardy-Weinberg equilibrium, and Popgene32 (Yeh *et al*., 2000) to examine heterozygosity.

Results and conclusions - Table 1 shows allelic and genotypic frequencies of the microsatellite at 3'-UTR *NRAMP1* according to the breed.

Table 1. Allelic and genotypic frequencies at 3'-UTR NRAMP1 microsatellite according to the breed.

	Allelic frequency			Genotypic frequency				
Breed	GT_{13}	GT_{14}	GT_{15}	GT_{13}/GT_{13}	GT_{14}/GT_{14}	GT_{13}/GT_{14}	GT_{13}/GT_{15}	GT_{14}/GT_{15}
Sarda (n=96)	0.874	0.063	0.063	0.823	0.042	0.010	0.094	0.031
Italian Brown (n=55)	0.973	0.018	0.009	0.964	0.018	-	0.018	-
Italian Friesian (n=36)	1.000	-	-	1.000	-	-	-	-

On the whole three alleles were found, GT_{13}, GT_{14}, and GT_{15}. GT_{13} showed the highest allelic frequency in all the breeds and was the unique allele in the Italian Friesian. GT_{14} and GT_{15} were found in the Sarda and in the Italian Brown. GT_{16} allele was not detected. Microsatellite extension and allelic frequency of the Italian Friesian are in accordance with those recorded by Paixao *et al*. (2006) in Holstein cattle reared in Brazil. Homozygous GT_{13}/GT_{13} was obviously the sole genotype of the Italian Friesian and the most representative genotype of the Italian Brown and Sarda. In the Sarda, four genotype variants other than GT_{13}/GT_{13} were found: GT_{14}/GT_{14}, GT_{13}/GT_{14}, GT_{13}/GT_{15} and GT_{14}/GT_{15}. As regards the Italian Brown, GT_{14}/GT_{14} and GT_{13}/GT_{15} were detected. Homozygous GT_{15}/GT_{15} was not detected in any sample. Sarda and Italian Brown populations were in Hardy-Weinberg disequilibrium. Observed heterozygosity was lower than the expected value both for the Sarda and the Italian Brown (Table 2).

Table 2. Genetic diversity at 3'-UTR *NRAMP1* microsatellite according to the breed.

Breed	Ho	He
Sarda (n=96)	0.135	0.228
Italian Brown (n=55)	0.018	0.054

Ho = observed heterozygosity; He = expected heterozygosity.

Results indicated that Sarda breed showed a high genetic variability, while as regards the two specialized breeds, Italian Brown and Italian Friesian, the high frequency of GT_{13} allele was probably caused by the long-term and intense genetic selection for the improvement of dairy productive traits. On the contrary, the variability of the Sarda, a local and non-specialized breed, was affected by the fact that cattle of this study belonged to farms situated in mountain areas and the herds were not submitted to crossbreeding with other specialized breeds, as it often happens in Sardinia with the Brown or Modicana. However, further investigations are required because of the small number of samples. Since the microsatellite at 3'-UTR *NRAMP1* was associated with resistance or susceptibility to certain intracellular pathogens, when the role of this gene in the complex mechanism of immunology will be totally elucidated, the knowledge of the allele at this locus could be suitable in future genetic schemes, in order to obtain natural resistant animals.

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