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GENEALOGICAL CONTROL OF BOVINE BREED RUBIA GALLEGA THROUGH 17 DNA MICROSATELLITE GENETIC MARKERS

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INTRODUCTION

The "Rubia Gallega" breed is the most characteristic cattle racial biotype of Spanish Norwest, representing the best example of the agricultural Galician profile and constituting an identity signal of Galicia landscape, customs or language. The census of registered pure-breed animals in the Herd Book managed by the National Association of "Rubia Gallega" Beef Cattle Breeders (ACRUGA) rises to more than 45,000 animals of which 60% are used as parents (26,846 females older than 24 months and 359 females older than 14 months).

Microsatellites markers have been widely used as a genetic markers in bovine population studies and pedigree verification (Visscher et al., 2002). These microsatellites are an important tool in population genetic studies. They are highly polymorphic, and present simple mendelian inheritance and codominance (segregation of homozygotes and heterozygotes) (Boichard et al., 1998).

The use of polymorphic microsatellites is a main tool to get a control of the whole genealogy of the race, to guarantee the genetic value of the animals, and therefore their economic value. Also, with the obtained data it will be possible to carry out a study of the genealogy of the reproducers.

The purpose of the present study was to examine genetic diversity of the "Rubia Gallega" cattle breed by means of microsatellite DNA polymorphisms. In addition, this study would bring out the variability at DNA level and genetic structure of the breed. We also estimated the identification paternity frequency in the population and the evaluation of potential use of these microsatellites in Paternity Tests and individual identification in "Rubia Gallega" breed.

MATERIAL AND METHODS

The experiment was conducted at Xenetica Fontao (Laboratory of Biotechnology and Molecular Genetics of Lugo (Spain). Blood samples and hair follicles were collected from 18,687 animals from Rubia Gallega cattle breed, located at distinct farms in Galicia (Spain), and registered pure breed in the Rubia Gallega Breeders Association (ACRUGA). Individual animals of the breed were chosen at random without consideration of the relationships among the animals. The microsatellites used on this work are recommended for bovine paternity tests

by the International Society of Animal Genetics (ISAG) in the Test of International Comparison of Bovine DNA (BM1818, BM1824, BM2113, CSRM60, ETH3, ETH10, ETH185, ETH225, ILSTS006, INRA005, INRA023, INRA063, SPS115, TGLA53, TGLA122, TGLA126 y TGLA227), organism of which east laboratory is institutional member, based on criteria established by Food and Agriculture Organization (FAO).

The extraction of DNA was carried out according to the protocols and using the reagents recommended by the maker, in function of the sample type. The extracted DNA has been amplified by means of PCR (Reaction in Chain of the Polimerase) for the analysis of 17 markers (following the guidelines of the ISAG) that are applicable to studies of molecular characterization, allowing the identification of the animals, as well as the correct assignment of the relationships of relationship in a reliable way. In the complex cases, in those that is not possible to elucidate among several proposed parents, the study is enlarged with the analysis of other 13 additional microsatellites, what the exclusion probability increases and, therefore of correct affiliation.

Microsatellite allele frequencies, observed (Hobs), and expected (Hexp) heterozygosity, Polymorphism Information Content (PIC) and exclusion of probabilities were calculated using the Cervus 2.0 software package

RESULTS AND DISCUSION

All 17 tested microsatellite markers were polymorphic. The number of alleles per locus varied from 5 to 24. Heterozygosities observed and expected ranged from 0.407 (SPS 115) to 0.870 (TGLA 53) and 0.409 (SPS 115) to 0.871 (TGLA 53) respectively.

Polymorphic information content was also high and only in one case (INRA 005) did not exceed value of 0.6. The highest value for PIC was found for TGLA 53 (0.860).

Average heterozygosity had a value of 0.709. With the exception of INRA063 and SPS115, the elected markers presented PIC values upper to 0.5, what made them highly informative, and specifically the markers TGLA53, BM2113, INRA023 and TGLA227 showed PIC values rising till 0.8 (Table 1).

These results indicate that the "Rubia gallega" breed has a high genetic variability. The studied loci did not show significant deviation from Hardy-Weinberg.

The presented set of microsatellites gives a powerful tool that can help in verification of the true relationships among animals. The 17 used markers present a probability of global exclusion (PE) of 99.9421 and 99.9997 for one or two progenitors respectively, what is very useful to carry out the filiation controls in this bovine breed.

The markers INRA063 and SPS115 presented low values of PIC (<0.5), and smaller exclusion probabilities. TGLA53 and BM2113 (with PIC values up to 0.8) are the markers that present bigger exclusion capacity, being of great utility in the control of genealogies.

The used markers also present the advantage that they allow to share and to compare the results obtained with those obtained for this or other bovine races, in any laboratory ISAG that has undergone the Test of International Comparison of Bovine.

Table 1. Microsatellite allele frequency, heterozygosity and Polymorphism Information Content (PIC) in Rubia Gallega cattle breed.

Locus	Allel	N	Hets	Hom	H(ob	H(es	PIC	PE(1	PE(2	H	Nullfr
BM1818	14	1868	1192	675	0,63	0,63	0,60	0,24	0,43	N	-0,00
BM1824	8	1868	1404	464	0,75	0,75	0,71	0,34	0,52	N	0,002
BM2113	11	1868	1613	255	0,86	0,85	0,84	0,55	0,71	N	-0,00
CSRM6	13	1868	1184	684	0,63	0,62	0,57	0,22	0,38	N	-0,00
ETH3	10	1868	1338	530	0,71	0,71	0,66	0,30	0,47	N	-0,00
ETH10	10	1868	1248	620	0,66	0,66	0,61	0,24	0,40	N	0,000
ETH185	20	1868	1289	579	0,69	0,68	0,64	0,28	0,46	N	-0,00
ETH225	10	1868	1455	413	0,77	0,77	0,74	0,38	0,56	N	-0,00
ILSTS0	13	1868	1437	431	0,76	0,76	0,73	0,38	0,56	N	-0,00
INRA00	5	1868	1156	712	0,61	0,61	0,54	0,19	0,33	N	0,000
INRA02	13	1868	1575	293	0,84	0,84	0,82	0,51	0,68	N	0,000
INRA06	10	1868	863	1005	0,46	0,47	0,39	0,11	0,22	N	0,008
SPS115	9	1868	761	1107	0,40	0,40	0,39	0,09	0,24	N	0,002
TGLA53	21	1868	1626	242	0,87	0,87	0,86	0,59	0,74	N	0,000
TGLA12	24	1868	1541	326	0,82	0,82	0,80	0,48	0,66	N	0,001
TGLA12	10	1868	1260	608	0,67	0,67	0,62	0,25	0,41	N	0,003
TGLA22	14	1868	1568	300	0,83	0,83	0,81	0,50	0,67	N	-0,00

In the reduced number of cases in which it is not possible to exclude between two or more possible progenitors the exclusion capacity has been increased with the analysis of an additional panel with 13 microsatellites markers, elected of the list proposed by the ISAG: (CSSM66, ETH152, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL15, ILSTS005, INRA032, INRA035, INRA037, MM12)

CONCLUSIONS

The markers are highly informative and can be used for characterization of domestic animal biodiversity. The obtained results in the present study lead us to conclude that the use of parentage verification based in a DNA test, in order to prove the accuracy of genealogical records is justified. This type of analysis will also be useful in cases of legal disputes concerning the ownership of a particular animal.

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