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Research Article

NOVEL BREED-SPECIFIC ALLELES IDENTIFIED BY MICROSATELLITE MARKERS IN SHEEP BREEDS

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Abstract: Breed-specific identification tools are very much essential for better livestock management, to promote conservation and preservation of livestock genetic diversity. Microsatellites are markers of choice for breed characterisation and differentiation. This study is aimed to characterize and to identify breed-specific alleles in Madras Red and Mecheri sheep breeds by using FAO recommended microsatellite markers. DNA was isolated by a rapid, non-enzymatic method using non-ionic detergent from 50 unrelated animals of each breed. A total of 10 microsatellite markers are used for PCR amplification which is followed by polyacrylamide gel electrophoresis and silver staining. The silver-stained gels were analysed by Diversity Database software for sizing of various allelic products in both the breeds of sheep studied. Breed-specific alleles were observed in all *loci* in both the breeds, except Oar FCB 128 and TGLA 377 which showed breed-specific alleles only in Madras Red sheep. Such breed-specific alleles and their frequencies obtained in this study can be efficiently used to differentiate Madras Red and Mecheri sheep at DNA level.

Keywords: *Microsatellite, Breed-specific alleles, Genetic diversity, Madras Red sheep, Mecheri sheep, Breed Identification and Characterisation*

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Introduction

The genetic characterisation of indigenous livestock breeds is the first step to safeguard our valuable germplasm for effective and meaningful conservation. A livestock breed can be defined as a homogenous group with unique and identifiable phenotypic features that distinguish it from other subgroups within the same species [1]. Breeds can therefore be regarded as the unit of management and conservation. Hence the development of breed-specific identification tools is very much essential for better livestock management, to promote conservation and preservation of livestock genetic diversity [2]. Due to the tremendous progress in the field of molecular biology, new class of markers called molecular markers has emerged during the last few decades. Among which, microsatellites are the markers of choice as they are abundantly distributed throughout the mammalian genome and of easy genotyping. They have a large number of alleles, a high level of heterozygosity and are inherited in the Mendelian fashion [3]. The microsatellites are highly polymorphic in nature and due to their high mutation rate and co-dominant nature that allow the estimation of within and between breed genetic diversity, microsatellite markers are used to differentiate the livestock breeds [4]. The purpose of this study was to characterise Madras Red and Mecheri, the most prominent meat type sheep breeds of Tami Nadu based on microsatellite polymorphism and to find out the genetic signature at molecular level through identification of breed-specific alleles. Breed-specific alleles are alleles scored for the molecular markers which are specific to a particular breed. This study will be of immense use in identifying genetic uniqueness of the breed, tracing the evolutionary origin and formulating effective conservation strategies for genetic diversity within and between breeds.

Materials and Methods

Blood samples were collected from 50 animals in each Madras Red and Mecheri sheep breeds located at Livestock Research Station, Kattupakkam, Kanchipuram district and Mecheri Sheep Research Station, Pottaneri, Salem district. The animals were selected randomly from each breed and were unrelated by ancestry. Genomic DNA was isolated by a rapid non-enzymatic method [5]. A total of 10 microsatellite markers were genotyped on 50 DNA samples of Madras Red and Mecheri sheep breeds [Table-1]. The microsatellite markers were selected as per the guidelines of Food and Agriculture Organisation of United Nations. The Polymerase chain reaction (PCR) amplification were carried out in a 20µl final reaction volume containing at least 20-50 ng of genomic DNA, 20 picomoles of each primer, 1.5mM $MgCl_2$, 100µM dNTPs, 0.25U *Taq* polymerase and 10x buffer. The PCR protocol was as follows: 3 min at 94°C followed by 30 cycles of 1 min at 94°C, 1 min at annealing temperature (varies for primers) and 1 min at 72°C. The last elongation step was for 10 min. After confirmation of amplification on 2% agarose gel, PCR products were electrophoresed on 6% denatured polyacrylamide gel and visualized by silver staining [6] along with a single stranded 10 bp DNA ladder (Invitrogen, USA) as marker. Estimation of allele size was done by using Diversity Database software (Bio-Rad, USA).

Results and Discussion

The most powerful markers for distinguishing among populations would be those that are fixed for different alleles in different breeds. In the present study, all the ten microsatellites showed breed-specific alleles with varying frequencies ranging from 0.0116 to 0.3974. The details on the breed-specific alleles and their frequencies are furnished in [Table-2]. The BM 8125, CSSM 31, Oar AE 129, Oar CP 34, OAR HH 35, Oar JMP 29, Oar JMP 8 and RM 4 gave breed-specific alleles for both Madras Red and Mecheri breeds. But the Oar FCB 128 and TGLA 377 markers gave breed-specific alleles in Madras Red breed only which are critical in terms of conservation. The BM 8125 showed two alleles specific to Madras Red only and these were 96 and 112 bp with similar frequency (0.0384) of occurrence and in Mecheri, breed-specific alleles was found to be 124 bp with a frequency of 0.0116. The CSSM 31 exhibited six breed-specific alleles specific to Madras Red

Table-1 *Primer sequence, location on chromosome number of alleles and annealing temperature of sheep microsatellite loci*

Locus	Primer sequence	Chromosome Number	Annealing temperature
BM 8125	P_1: CTCTATCTGTGGAAAAGGTGGG	17	55
	P_2: GGGGGTTAGACTTCAACATACG		
CSSM 31	P_1:CCAAGTTTAGTRACTTGTAAGTAGA	23	55
	P_2: GACTCTCTAGCACTTTATCTGTGT		
Oar AE 129	P_1: AATCCAGTGTGTGAAAGACTAATCCAG	5	60
	P_2: GTAGATCAAGATATAGAATATTTTTCAACACC		
Oar CP 34	P_1: CTGAAGAATGTGATATGTTCAGG	5	63
	P_2: GGGACAATACTGTCTTAGATGCTGC		
Oar FCB 128	P_1: CAGCTGAGCAACTAAGACATACATGCG	2	63
	P_2: ATTAAAGCATCTTCTCTTTATTTCCTCGC		
OAE HH 35	P_1: AATTGCATTCATATCTT"TAACATCTGGC	4	63
	P_2: ATGAAAATATAAAGAGAATGAACCACACGG		
Oar JMP 29	P_1: GTATACACGTGGACACCGCTTTGTAC	24	55
	P_2: GAAGTGGCAAGATTCAGAGGGGAAG		
Oar JMP 8	P_1: GGGATGATCTTCTGTCCAAATATGC	6	63
	P_2: ATTTGCTTTGGCTTCAGAACCAGAG		
RM 4	P_1: AGCAAAATATCAGCAAACCT	15	55
	P_2: CCACCTGGGAAGGCCTTTA		
TGLA 377	P_1: ACTGTCATTATCTTCCAGCGGAC	2	55
	P_2: ATCTCTGGTTGAAATGGCCAGCAG		

Table-2 *The breed-specific alleles and their frequencies*

Locus	Madras Red		Mecheri	
	Allele size (bp)	Frequency	Allele size (bp)	Frequency
BM 8125	96 112	0.0384 0.0384	124	0.0116
CSSM 31	126 128 138 156 158 162	0.0250 0.0125 0.0125 0.0625 0.0625 0.0375	132 140	0.0166 0.0166
Oar AE 129	162 164 166 167 168	0.0119 0.0238 0.0119 0.0119 0.0119	150 154	0.2209 0.0930
Oar CP 34	116 119	0.0384 0.2564	111 113 120 122 127 145 147	0.0450 0.0450 0.0757 0.151 0.0450 0.1515 0.0454
Oar FCB 128	106 111 114	0.0555 0.0666 0.0222	-	-
Oar HH 35	119 120 130	0.0975 0.1585 0.0121	124 128	0.2444 0.1777
Oar JMP 29	124 126	0.0217 0.0543	133	0.0333
Oar JMP 8	139	0.0119	119 121 123	0.0125 0.0370 0.0500
RM 4	146 148 150 151 152 153 154 156	0.0256 0.0512 0.1538 0.0641 0.3974 0.0384 0.2435 0.0256	134 136 138 140 142 144	0.0348 0.1162 0.2325 0.3488 0.2325 0.0348
TGLA 377	92 94 105	0.0172 0.0172 0.012	-	-

which were 126, 128, 138, 156 and 162 bp with a frequency of 0.0250, 0.0125, 0.0125, 0.0625, 0.0625 and 0.0375 respectively. While in Mecheri, two alleles of size 132 and 140 bp were found to be specific with a frequency of 0.0166 each. At Oar AE 129 locus, 162, 164, 166, 167 and 168 bp alleles were breed-specific in Madras red with almost similar frequencies. In Mecheri, of the two specific alleles (150 and 154 bp), 150 bp allele was found to be predominant. The Oar CP 34 locus showed two breed-specific alleles in Madras Red (116 and 119 bp) while in Mecheri, a greater number of breed-specific alleles were observed. Oar HH 35 locus produced three specific alleles to Madras Red breed and two in Mecheri. There were two alleles specific to madras Red (124 and 126 bp) and only one (133 bp) in Mecheri sheep with respect to Oar JMP 29 locus. On the contrary, Oar JMP 8 locus had only one specific allele in Madras Red and three in Mecheri.

A total of eight exclusive (146, 148,150, 151, 152, 153, 154 and 156 bp) alleles at RM 4 locus was found in Madras Red with the frequencies ranging from 0.0256 to 0.3974. In Mecheri, there were six specific alleles of sizes 134, 136, 138, 140, 142 and 144 bp with a frequency range of 0.0348 to 0.3488. Out of the ten *loci* studied, Oar FCB 128 and TGLA 377 exhibited exclusive alleles in Madras Red sheep. While, there was no such specific alleles noticed at these two *loci* in Mecheri sheep. The allele sized were 106 (0.0555), 111 (0.0666) and 114 bp (0.2220) for Oar FCB 128 and 92, 94 and 105 bp (0.0172) alleles for TGLA 377 locus. Some microsatellites showed many specific alleles in one breed and few in another; for example, CSSM 31 and Oar AE 129 were found to be highly polymorphic in Madras Red and Oar CP 34 in Mecheri with comparatively more breed-specific alleles. The finding of the present study was comparable with the other previous studies. In Swiss sheep breeds, ILSTS 005, Oar CP 20, TGLA 73 and URB 058 generated breed-specific alleles and their frequencies reached almost 20 per cent [7]. In a similar study, number of alleles at cattle microsatellites namely, ETH 225, ILSTS 005, ILSTS 011, ILSTS 033 and INRA 035 *loci* in Madras Red, Mecheri and Nilgiri sheep were 5, 8 and 6; 11, 8 and 6; 8, 11 and 6; 10, 9 and 10 and 12, 8 and 9 respectively. The number of breed-specific alleles observed in Madras Red were 8 (175, 179, 181, 185, 193, 195, 211 and 244 bp) for ILSTS 005, one (287 bp) for ILSTS 011, 5 (155, 157, 179, 182 and 191 bp) for ILSTS 033 *loci* and no breed-specific alleles for ETH 225 locus. In Mecheri sheep, breed-specific alleles were 143,150,157, 164, 171 and 179 bp for ETH 225,242 bp for ILSTS 005, 263, 275, 278, 288, 289, 316 and 333 bp for ILSTS 011. The frequencies of these breed-specific alleles were found to be ranging from 0.028 to 0.348 [8]. Perusal of literature did not reveal any such reports for comparison with the present findings. However, breed-specific alleles had also been reported in other species of livestock at various microsatellite *loci* such as goats [9] and cattle [10] and buffalo [11].

Conclusion

Breed-specific alleles and their frequencies obtained in this study can be efficiently used to differentiate Madras Red and Mecheri sheep at DNA level. But it is major challenge to develop a general method to discriminate breeds based on breed-specific alleles in livestock. It requires a large number of breed-specific alleles to be genotyped on a sufficiently large population. However, with the advancement of DNA technology in nowadays, this is not impossible. Breed identification based on breed-specific alleles of various molecular markers would be an easy, rapid, economic, and reliable method in future.

Application of research: This study may be applied to identify genetic signature of the livestock breeds and characterisation and analysis of relationship among the breeds of various livestock species.

Research Category: Animal Genetics and Breeding

Abbreviations: FAO: Food and Agriculture Organisation of United Nations;
PCR: The Polymerase chain reaction

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Study area / Sample Collection: Livestock Research Station, Kattupakkam, Kanchipuram district and Mecheri Sheep Research Station, Pottaneri, Salem

Breed name: Mecheri Sheep

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tirunelveli, 627 358, Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051, Tamil Nadu, India.
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