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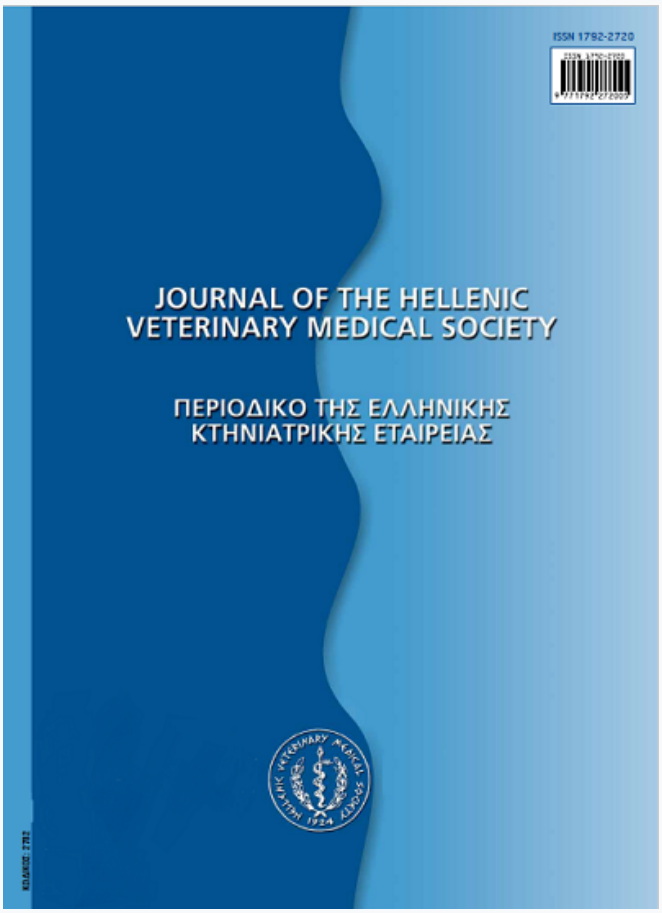

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Research article
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Molecular Diversity Analysis of Jattal and Dera Din Panah Goat Breeds of Pakistan using Microsatellite Markers

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ABSTRACT. This paper evaluates the genetic diversity of goat breeds in Pakistan, a country blessed with a wide range of goats spread throughout with distinct features contributing to a rich Animal Genetic Resource. The genetic diversity of two goat breeds (Jattal and Dera Din Panah) was assessed with 25 animals representing the two breeds using 16 microsatellite markers. The mean observed and expected heterozygosity of both goat breed populations were observed as 0.83+0.21. The average number of observed alleles was 3.6+1.6 for all loci. The mean polymorphic information content for a goat breed was 0.45, indicating the usefulness of markers panel. Highest Nei's standard genetic distance (Ds) value of 0.0612 was observed between Jattal and Dera Din Panah goats, and the mean Fst value was 0.013. The measures of genetic variation revealed a good scope for effective improvement, conservation, and designing national breeding policies, in future, for Pakistan goat breeds.

Key Words: Microsatellite markers, genetic diversity, goat breeds, Pakistan

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INTRODUCTION

Pakistan, which is the third largest goat producing and the tenth largest goat producing country in the world, has 30 goat breeds (*Capra hircus*) that play a major role in the economy of poor farmers (Khan et al. 2008). For the effective utilization of this rich Animal Genetic Resource of Pakistan through suitable breeding and conservation strategies, characterization and evaluation of genetic variations among these breeds are necessary. The first step in conservation of biodiversity identifies the current genetic variability and then build a pool of genetic information for conservation. The current study was designed to genetically characterize the selected two goat breeds of Pakistan using microsatellite markers. Microsatellite loci are currently used, due to its high degree of polymorphism, and it is a most powerful tool for inferring genetic diversity (Bruford and Wayne 1993; Marikar and Mustafa 2013). Microsatellites, also called as short tandem repeats (STR), are among the preferred methods of genetic assessment because of their abundance, extremely high polymorphism, low mutation rate, ease of amplification through PCR, and small product size (Jouquand, Priat et al. 2000; Teneva, Todorouska et al. 2005).

This paper reports the first molecular investigation of two endemic breeds of goats in Pakistan region in which Jattal and Dera Din Panah goat breeds were selected for characterization. Dera Din Panah goat breed is an inhabitant of Muzaffargarh and Multan districts of Punjab, while Jattal goat locates in Azad Jammu and Kashmir (1985). These two breeds are very difficult to get in to the research because both are living in the Kashmir region and Punjab and less attention was given. Therefore the main objective of this study was to characterize and observe genetic variation in goat breeds through microsatellite markers for making effective strategies for the conservation and proper management of the biological resources of Pakistan, mainly because they are endemic and endangered species.

MATERIALS AND METHODS

Animal selection and DNA Extraction

Ten (10) ml of blood samples were collected aseptically from the jugular vein of 25 animals, each from Jattal and Dera Din Panah goat breeds, into 15 ml falcon tubes containing 200 µL ethylenediamine tetraacetic acid (EDTA). Selected animals were unrelated as they were collected from breeding areas of Jattal breed in Mirpur and Kotli districts in Azad Jammu and Kashmir region close to boarder area while Dera Din Panah samples were collected from Government Livestock Farm at Rukh Khery Wala District Layyah in Punjab province.. Genomic DNA was extracted using an inorganic method of extraction in the molecular laboratory (Sambrook and Russell 2001). The final concentration of extracted DNA was made to 50 ng/uL through spectrophotometry and 0.8% agarose gel electrophoresis.

Markers Selection and Genotyping

Sixteen (16) microsatellite markers (FAO recommended) were selected. Table 1 presents details of all microsatellite loci. All microsatellites were optimized for PCR amplification through BioRad thermo cycler using reaction mixture of 25 uL containing 100 ng DNA, 50 mM KCl, 10 mM Tris-HCl, 2 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 pmol/ uL of forward and reverse

Table 1. I.Microsatellite markers description; marker, product size and chromosome number

Marker	ASR	Chromosome
MAF70	127-188	4
OarFCB11	130-161	2
OarAE101	100-134	6
MAF33	113-147	9
OarFCB128	106-136	2
OarFCB304	123-201	19
OarHH47	138-177	18
OarVH72	122-150	25
BM0757	138-189	9
INRA32	150-194	11
BM1818	259-313	32
ILSTS011	300-382	24
MM12	93-131	9
ETH152	181-236	5
INRA032	194-230	11
OarFCB48	104-187	17

primers, and 0.15 uL of 5U Taq polymerase (Fermentas, USA). Touch-down PCR was used for amplification. The initial denaturing at 950C for 4 minutes was followed by 35 cycles each for 30 seconds at 940C for denaturation, 45 seconds at 640-540 C for annealing, and 45 seconds at 720 C for extension. This was followed by 10 minutes at 720C for the final extension. The products were electrophoresed on 12% non-denaturing polyacrylamide gel in 1X TAE buffer at 120 volts for 7 hours.

Statistical Analysis

The results of polyacrylamide gel electrophoresis were analyzed by the relative flow method. Statistical analysis was performed for each microsatellite marker to calculate the genetic variability measures such as the number of alleles, expected and observed heterozygosity, homozygosity, and Polymorphic Information Content (PIC). Genetic distances between the breeds were determined according to Nei (Nei 1973). The unweighted pair-group method (UPGM) helped to make the genetic distance dendrogram, while POPGENE 1.31 and POWER STAT software were used for calculations (Yeh and Yong 1999).

RESULTS

A total of 59 alleles were found in the 16 loci in all two breeds as a whole. The number of alleles per locus ranged from 2 (OarAE101, MAF33, OarFCB128, OarHH47, BM1818, and INRA032) to 6 (MAF70, INRA32, ETH152, and OarFCB48) and the observed number of alleles (na) for all populations per loci was 3.6+1.6 (Table 2). The observed heterozygosities in all studied breeds ranged from 0.32 (OarFCB11) to 1 (MAF70, OarFCB304, OarVH72, ILSTS011, MM12, ETH152, and OarFCB48) with an average of 0.83+0.21, while the expected heterozygosities throughout all the breeds ranked between 0.48 (OarFCB128) and 0.0.79 (OarFCB48); and the average was 0.61+0.12. The average heterozygosity in Jattal and D.D.P. was observed as 0.543. The average number of alleles per breed was observed from between 2.8 (Jattal and Dera Din Panah) (Table 3), and the average PIC value for both goat populations was 0.45. Goat populations revealed a significant genetic distance from each other (Figure 1) (Nei 1978).

Table 2. Summary F-Statistics and Gene Flow for All Loci in Jattal and Dera Din Panah goat breeds

Marker	Fis	Fit	Fst	Nm
MAF70	-0.5133	-0.5078	0.0036	68.8333
OarFCB11	0.5077	0.5077	0	****
OarAE101	-0.5651	-0.5422	0.0146	16.8611
MAF33	-1	-1	0	****
OarFCB128	-0.5612	-0.5606	0.0004	624.5
OarFCB304	-0.3998	-0.3877	0.0086	28.8065
OarHH47	-0.7707	-0.7241	0.0263	9.2656
OarVH72	-1	-1	0	****
BM0757	-0.5978	-0.5595	0.024	10.1825
INRA32	****	****	0	****
BM1818	-0.7707	-0.7241	0.0263	9.2656
ILSTS011	-0.4351	-0.4314	0.0026	96.7778
MM12	-0.5567	-0.5333	0.015	16.3878
ETH152	-0.3617	-0.2652	0.0709	3.2786
INRA032	-0.7214	-0.7207	0.0004	624.5
OarFCB48	-0.3691	-0.3691	0	****
Mean	-0.5158	-0.4949	0.0138	17.8172
St. Dev				

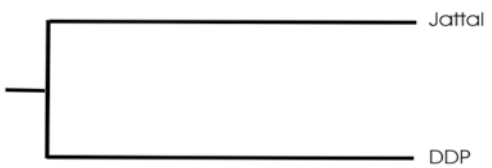

Figure. 1. Dendrogram Based Nei's (1972) Genetic distance: Method = UPGMA, Modified from NEIGHBOR procedure of PHYLIP Version 3.5

The genetic identity of Jattal and Dera Din Panah goat was 0.940 (Table 4) (Nei 1978), and the genetic distance between two goat breeds was 0.0612 with more similarity between Jattal and D.D.P goat breeds. The dendrogram based on Nei's (1978) genetic distance using UPGMA method indicated the close relationship between Jattal and D.D.P. goat breeds; however, these goat breeds were distinct (Figure 1).

Table 3. Summary of Genetic Variation Statistics and Heterozygosity Statistics for All Loci

Marker	Na	Ne	I	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het_Het
MAF70	4	2.9691	1.2316	0	1	0.3301	0.6699	0.6632	0.6608
OarFCB11	3	2.3191	0.9308	0.72	0.28	0.4255	0.5745	0.5688	0.5688
OarAE101	2	1.9716	0.6859	0.24	0.76	0.5022	0.4978	0.4928	0.4856
MAF33	2	2	0.6931	0	1	0.4949	0.5051	0.5	0.5
OarFCB128	2	1.9992	0.6929	0.22	0.78	0.4952	0.5048	0.4998	0.4996
OarFCB304	4	3.5791	1.3223	0	1	0.2721	0.7279	0.7206	0.7144
OarHH47	2	1.9501	0.6803	0.16	0.84	0.5079	0.4921	0.4872	0.4744
OarVH72	2	2	0.6931	0	1	0.4949	0.5051	0.5	0.5
BM0757	3	2.1088	0.8475	0.18	0.82	0.4689	0.5311	0.5258	0.5132
INRA32	1	1	0	1	0	1	0	0	0
BM1818	2	1.9501	0.6803	0.16	0.84	0.5079	0.4921	0.4872	0.4744
ILSTS011	4	3.3179	1.2721	0	1	0.2943	0.7057	0.6986	0.6968
MM12	4	2.8752	1.1754	0	1	0.3412	0.6588	0.6522	0.6424
ETH152	6	4.771	1.6164	0	1	0.2016	0.7984	0.7904	0.7344
INRA032	2	1.9992	0.6929	0.14	0.86	0.4952	0.5048	0.4998	0.4996
OarFCB48	4	3.7092	1.3466	0	1	0.2622	0.7378	0.7304	0.7304
Mean	2.9375	2.5325	0.9101	0.1762	0.8237	0.4434	0.5566	0.5511	0.5434
St. Dev	1.2894	0.9352	0.3951	0.2864	0.2864	0.1825	0.1825	0.1806	0.1761

Table: 4. Nei's Unbiased Measures of Genetic Identity and Genetic distance (Nei1978). Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Population ID	Jattal	D.D.P
Jattal	****	0.9784
D.D.P	0.0218	****

DISCUSSION

Studies on genetic characterization of goat breeds are extremely limited in Pakistan. Most of the selected microsatellite markers showed polymorphism, indicating their usefulness. The average heterozygosity of Pakistani goat was 0.543, which is lower than Chinese goat populations 0.677 (Wang, Yang et al. 2006) and 0.77 (Yang, Zhao et al. 1999), and higher than Indian goat populations 0.426 (Fatimaa, Bhonga et al. 2008; Rout, Joshi et al. 2008).

The observed heterozygosity in Jattal and Dera Din Panah goat (0.822, 0.825) breeds was higher than expected heterozygosity values of goats (0.559, 0.549), indicating no overall loss of heterozygosity (allele fixation) in the studied breeds. Mean values of Fis (heterozygote deficit) were -0.515 for goat breeds are low, representing lower inbreeding coefficient between breeds, which is evident from the home

track distance of the studied breeds in contrast to the positive Fis values (high level of inbreeding) in Italian goat breeds (Bozzi, Degl'Innocenti et al. 2009).

Polymorphism information content (PIC) is another good indicator of marker efficiency for genetic studies. In this study, the average PIC value of 16 loci was 0.45 in Jattal and Dera Din Panah goat breeds, while 0.53, which was lower than Indian domestic goats (0.60) (Pandey, Sharma et al. 2010), Chinese (0.62), and Saanen (0.57) goat breeds, but greater than Korean goats (0.35) (Kim, Yeo et al. 2002). The high average PIC values of the panel of microsatellites used for all breeds supported the appropriateness of markers for genetic diversity analysis in Pakistan goat breeds.

Genetic distance between the studied goat breeds showed genetic differentiation among them and reflected the morphological characters as well e.g. Jattal breed is meat type small breed with medium size ears, hair present on chin, spiraled horns in males but smooth in females, small udder and teats while Dera Din Panah is a large black color dairy goat breed having long hair, long hanging and twisted ears and long spiral horns along with well-developed udder and teats. Further, the dendrogram explains the close relationship between Jattal and Dera Din Panah goats, revealing a considerable genetic relatedness.

This study concludes about a high genetic diversity in Pakistani goat breeds and confirms that genotyping through microsatellites is an effective tool for genetic evaluation of different breeds. This microsatellite panel can be used on other domestic and wild goat breeds of Pakistan, and the variation in the values of this study with other reports may be due to the difference of markers, breeding plans, laboratory techniques, and sample size.

CONCLUSION

Future researchers can use these results as a basic guide for better understanding of the genetic relationship and breed differences in goat breeds for making prospective breeding policies and conservation plans to protect any loss in allelic variation in goat breeds in the country.

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