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Genetic Diversity of the Karayaka Sheep Breed in Samsun, Turkey

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Abstract: The Karayaka sheep is the most populous sheep breed in the Black Sea region of Turkey and a significant portion of the breed (30-35%) has been raised for centuries in Samsun province. For this reason, we aimed to investigate the genetic diversity of Karayaka sheep raised in Samsun, Turkey. The blood samples used in this study were collected from unrelated animals and were analysed using nine microsatellite markers. A total of 72 alleles were determined with an average of eight alleles per locus. The highest number of alleles (12) was observed for locus BM1314, while the lowest (4) was observed for locus CSSM47. The expected heterozygosity value (He) ranged from 0.654 at locus BM757 to 0.867 at locus BM1314, with an average of 0.757, whereas the observed heterozygosity (Ho) ranged from 0.125 at locus CSSM47 to 0.438 at locus BM757 and HUJ616, with an average of 0.329. The Wright's fixation index (F_{IS}) values ranged from 0.360 at locus BM757 to 0.833 at locus OARFCB304, with an average of 0.572. The results obtained from this study showed that Karayaka sheep population has a high level of genetic diversity and the studied markers were highly informative.
Keywords: Karayaka sheep breed, Genetic diversity, Microsatellites, Turkey.

INTRODUCTION

Sheep breeding among small ruminants is one the most intensive fields of animal production in Turkey [1]. The Karayaka sheep, a major farm animal genetic resource, is the most populous and dominant breed in the mid Black Sea region of Turkey [2]. Among the most famous of meat sheep breeds [3], Karayaka is an important breed for meat production in the Black Sea region. The fact that this breed is lean-tailed and well adapted to the harsh and humid environment of the region makes it especially important [4].

According to TUIK (Turkish Statistical Institute), The sheep population of Turkey in 2016 was 30,983,933. Karayaka sheep are estimated to comprise 4–5 % of total sheep population in the country. In the 1983–2012 period, the number of Karayaka sheep decreased from 1,700,000 to 500,000-600,000 according to DAD-IS (Domestic Animal Information System). Therefore, since 2006, the Karayaka sheep has been protected as a pure breeding by Turkish Ministry of Food, Agriculture and Livestock.

In farm animals, genetic diversity has been identified using various molecular techniques. Microsatellites, which are useful genetic markers due to their wide distribution in genomes, high levels of polymorphism, codominant inheritance and easy genotyping, are widely preferred in studies determining genetic diversity in livestock species [5-11].

Several genetic characterization studies using microsatellite and other markers have already been reported for Turkish sheep breeds [12-15]. Das *et al.* [13] characterised the genotypes of some Turkish local sheep breeds including Karayaka using seven microsatellite markers, and found that the Fıs value was highest (0.353) in the Karayaka population, and that all populations were not in Hardy-Weinberg equilibrum. Yilmaz *et al.* [11] investigated the genetic diversity of nine Turkish sheep breeds including Karayaka using 18 microsatellite loci. They found that the 18 samples belonged to two populations, and the Karayaka sheep breed had high genetic diversity.

Until now, the genetic diversity of Karayaka sheep in Samsun, Turkey has not been reported. A significant portion of the Karayaka population (30–35%) has been raised in Samsun for centuries. It is therefore very important to collect information on the genetic diversity of the Karayaka sheep raised in the region. For this reason, we aimed to investigate the genetic diversity of Karayaka sheep in Samsun, Turkey using nine microsatellite markers.

MATERIALS AND METHODS

Sample collecting and DNA extraction

Blood samples were obtained from 86 unrelated Karayaka sheep reared in different regions of Samsun, Turkey. All samples collected into collection tubes containing Ethylene diamine tetra-acetic acid (EDTA, 0.5 mM, pH 0.8) and stored at -20 ^{o}C until DNA isolation. Genomic DNA was extracted from the whole blood using a genomic DNA extraction kit (IDPURE™ Spin Column, USA).

Microsatellite genotyping

All samples were amplified by PCR method for nine microsatellite loci presented in Table 1. PCR reactions were performed in a final volume of 20 µL, consisting of 10 µL of 2X AmpMasterTM Taq (GenALL®, Korean), 1 µL of (10 pmol/ µL) forward and reverse primers, 2 µL of total DNA (30–50 ng) with ultrapure water added to a the total volume of 20 µL. The microsatellite loci were amplified using a thermal cycler program, with an initial denaturation of 95 ^{o}C for 5 min, followed by 35 cycles of, 30 s at an optimized annealing temperature for each primer, with a final extension at 72 ^{o}C for 4 min.

Table-1: The microsatellite locus used in the present study

Locus Name	Primers (5’ Forward and 3’ Reverse)	ASR	CHR
BM757	**F:** TGG AAA CAA TGT AAA CCT GGG **R:** TTG AGC CAC CAA GGA ACC	176-200	9
BM6526	**F:** CAT GCC AAA CAA TAT CCA GC **R:** TGA AGG TAG AGA GCA AGC AGC	161-175	19
BM827	**F:** GGG CTG GTC GTA TGC TGA G **R:** GTT GGA CTT GCT GAA GTG ACC	204-224	3
BM1314	**F:** TTC CTC CTC TTC TCT CCA AAC **R:** ATC TCA AAC GCC AGT GTG G	137-169	22
BM8125	**F:** CTC TAT CTG TGG AAA AGG TGG G **R:** GGG GGT TAG ACT TCA ACA TAC G	116-122	17
CSSM47	**F:** TCT CTG TCT CTA TCA CTA TAT GGC **R:** CTG GGC ACC TGA AAC TAT CAT CAT	152-178	2
HUJ616	**F:** TTC AAA CTA CAC ATT GAC AGG G **R:** GGA CCT TTG GCA ATG GAA GG	115-154	13
MAF33	**F:** GAT CTT TGT TTC AAT CTA TTC CAA TTT C **R:** GAT CAT CTG AGT GTG AGT ATA TAC AG	121-141	9
OarFCB304	**F:** CCC TAG GAG CTT TCA ATA AAG AAT CGG **R:** CGC TGC TGT CAA CTG GGT CAG GG	150-188	19

ASR: allele size range (bp), **CHR:** chromosome.

PCR products were analysed 2 % agarose gels stained with EtBr (500 µ/ml in H_2O). Sixteen samples were chosen for microsatellite genotyping of each locus. PCR fragments were genotyped by capillary electrophoresis in Qsep100™ DNA fragment analyzer (BiOptic Inc., USA). Allele sizing was performed using BiOptic software v 1.5.4.2654.

DATA ANALYSIS

Allele number (Na), and allelic richness (Ar) for each locus were calculated using the FSTAT version 1.2 software package [16]. Observed and Expected heterozygosity values for each locus were calculated using GENETIX version 4.05. [17]. The polymorphism Information Content (PIC) was estimated according to Botstein *et al*. using Cervus version 3.0.7 [18].

Inbreeding coefficient (Fis) values from Wright’s F-statistics for each locus were calculated according to Weir and Cockerham, using FSTAT version 1.2 software package [16]. The Fis values, which are one of the measurement methods used to determine the population from Hardy-Weinberg equilibrium, were calculated for each locus to the 95 % confidence interval, with 10 bootstraps. Significant levels of departure from HWE were determined using GENETIX version 4.05 [17] by making 1,000 permutation tests.

RESULTS AND DISCUSSIONS

This study provides information about the genetic diversity of the Karayaka sheep breed raised in the Samsun province of Turkey. Genetic diversity parameters for the Karayaka sheep are shown in Table 2. The polymorphism information content (PIC) is an index that determines the usefulness of any genetic marker [32]. The PIC values of the markers used in the present study were between 0.359 and 0.855 with an average value of 0.686±0.153. Microsatellite markers with PIC values higher than 0.50 were named as polymorphic markers [32]. In this regard, most of the used markers in the present study were highly polymorphic, with the exception of CSSM47 locus (0.359), which was moderately informative (0.25 < PIC < 0.50).

Table-2: Genetic diversity parameters calculated for nine microsatellite loci

Locus	N	Na	ASR	Ar	Ho	He	PIC	F_{IS}	HWE
BM757	16	7	186/208	5.659	0.438	0.654	0.570	0.360***	***
BM6526	16	6	166/182	5.639	0.313	0.758	0.723	0.608***	***
BM827	16	11	210/242	9.141	0.375	0.815	0.794	0.562***	***
BM1314	13	12	140/182	11.138	0.308	0.867	0.855	0.668***	***
BM8125	16	10	104/138	8.520	0.438	0.795	0.774	0.475***	***
CSSM47	16	4	132/172	3.375	0.125	0.707	0.359	0.704***	***
HUJ616	16	10	124/150	8.539	0.438	0.822	0.801	0.493***	***
MAF33	15	6	126/146	5.389	0.400	0.684	0.633	0.444***	***
OarFCB304	16	6	170/204	5.543	0.125	0.707	0.664	0.833***	***
Mean SD		8 2.78		6.994 2.447	0.329 0.126	0.757 0.073	0.686 0.153	0.572 0.147	

N: number of sample, Na: number of alleles, Ar: allelic richness, ASR: allele size range (bp), Ho: observed heterozygosity, He: expected heterozygosity, PIC: polymorphic information content F_{IS}: inbreeding coefficient, SD: standart deviation
***P<0.001

A total of 72 alleles were determined, with an average number of alleles of 8.00±2.78 for nine microsatellite loci. The highest and lowest allele numbers observed were 12 at the BM1314 locus and four at the CSSM47 locus, respectively. Similar results have also been shown in previous studies [19-21].The mean number of alleles (MNA) obtained in this study was lower than in previous studies [11, 22], but higher than for some of the breeds in other studies. Among the possible reasons for high or low allele number, the number of breeds, samples, and loci, etc used may be shown. When we compared the MNA to values obtained from some European and Asian sheep breeds [6, 19-21, 23], we found the number to be similar or higher in the Karayaka breed. Despite the low sample number in the present study, we found that Karayaka sheep breed exhibited considerable allelic diversity. This diversity is probably due to the fact that the domestication centre of sheep (*Ovis aries*) is very close to Turkey [24]. The number of different alleles can vary depending on the sample size. To account for this possibility, we used the allelic richness parameter, a measure of genetic diversity indicates [25] that considers the sample size [26] . The average allelic richness was found to be 6.994±2.447, ranging between 3.375 in CSSM47 to 11.138 in BM1314. For observed heterozygosity (Ho), the highest value was found to be 0.438 at the HUH616, BM8125, and BM757 loci and the lowest values were found to be 0.125 at the CSSM47 and OarFCB304 loci. For expected heterozygosity (He), the highest was 0.867 at the locus BM1314 loci and the lowest value was 0.654 at the BM757. The average values of Ho and He were found to be 0.329±0.126 and 0.757±0.073, respectively.

A higher value for H_o than for H_e indicates decreased in heterozygosity or increased homozygosity. The mean expected heterozygosity, a good indicator of genetic variability in a population [27], was higher in this study than in some studies [19, 23].

The F_{IS} values for all loci varied between 0.360 at BM757 to 0.833 at OarFCB304 with an average value of 0.572±0.147. The high or positive Fis values found in the present study result from uncontrolled breeding, low herd size and, some conditions that have the effect of increasing inbreeding [21, 28, 29]. Fis values calculated for the Karayaka sheep breed by other investigators were also positive, similar with those in our study while were lower than those reported by previous studies [5, 11, 30, 31]. The calculated Fıs values for all loci were significant (P < 0.001). For this reason, departures from Hardy-Weinberg equilibrium were found to be statistically significant (P < 0.001) at all studied loci. In addition to a high level of inbreeding, deviations from Hardy-Weinberg equilibrium may be caused by some other factors, such as the presence of null alleles, a small sample size, a deficiency in heterozygosity etc. The most common

reason for deviations from HWE is the presence of null alleles. According to results obtained in the present study, the Fis values for all loci used in the present study show that pure breeding has been maintained in flocks of Karaya sheep in Samsun, Turkey.

CONCLUSIONS

According to the obtained results, we can say that for nine microsatellite loci, sheep of the Karayaka breed raised in the Samsun province of Turkey have high genetic diversity and a high inbreeding coefficient (Fis). This high level of genetic variation can be useful for breeding programs. On the other hand, the results also suggest that some steps should be taken to decrease the level of inbreeding. The high levels of inbreeding could be a result of pure breeding programs carried out over long time periods by farmers in Samsun city. The microsatellite markers used in present study were sufficiently polymorphic, and thus they can be used in future genetic diversity studies. A limitation of the present study is that the number of studied loci is low, as a result of financial limitations and the expense of this kind of study. Even so, we think that the obtained results are important for conservation studies in Turkey.

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