Journal of Animal Research: v.9 n.1, p. 27-33. February 2019

DOI: 10.30954/2277-940X.01.2019.4

SSR based Characterization of Indigenous Harnai Sheep Breed of Balochistan

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Received: 23 Nov, 2018 **Revised:** 03 Jan., 2019 **Accepted:** 04 Jan., 2019

ABSTRACT

This study was on the molecular characterization of Harnai sheep breed in Balochistan. A set of (n=16) ovine specific SSR markers, recommended by FAO, was used on (n=50) blood samples from unrelated animals of Harnai sheep breed from their breeding tract. Various genetic parameters were observed using Pop gene software. A total of 74 alleles were found on 13 loci. The finding values for observed number of alleles (Na), effective number of alleles (Ne) and Shannon's Information index (I) the average values were found along with standard deviation to be 2.448±0.869, 1.7050.604 and 0.5890.357 respectively, further more, the mean values of observed heterozygosity (Obs_Het) expected homozygosity (Exp._Hom), expected heterozygosity (Exp_Het), effective number of allele (Ne) average Heterozygosity (Ave Het) were found to be 0.598±0.299, 0.366±0.284, 0.602±0.238, 0.363±0.219, 0.347±0.209 and 0.347±0.209, respectively. The value of F-statistic ranged from 0.2851 to 0.9132 for different microsatellite markers with an average of 0.515±0.021. Majority of the markers showed higher than average expected reduction in heterozygosity. The standard errors were generally low, which indicated that homozygosity prevails in the population under study. This might be due to intense inbreeding in this flock of Harnai sheep.

Keywords: SSR Markers, Harnai Sheep, Balochistan, Characterization

Balochistan, area wise, is the largest province of Pakistan, with the total area of 348,189 sq.km, and accounting for about 44% of the whole country's land area. This province is mainly outside the monsoon region. Irrigated land is uncommon, only 5% of contributes almost 50% share towards agriculture in the economy of Balochistan, this makes about 52% share in the provincial Gross Domestic Product (Vatankhah, 1998).

Rangelands are the major feed-source for these animals and about 90% of the feed-requirements are coming from the rangelands (Khan, 1994).

Livestock rearing on the common and free/open access rangelands is the major occupation of the rral people of Balochistan. Small ruminants (sheep & goats) are common livestock in the province and more than 85% people of Balochistan driving their income from these sources (Kakar *et al*., 2009). These animals are reared, almost by all kind of farmers and livestock traders. There are three types of pastoralism in the province viz: Nomadic, Transhumant and Sedentary pastoralism (Ahmad *et al*., 2012).

Balochistan possesses some well-known sheep breeds these local breeds, Government as well as the farmers of the area are trying to conserve these local breeds (Mirza and Akhter, 2007). Bibrik (Beverigh), Balochi, Harnai, Rakhshani, and Mengaliare five main sheep breeds (Tariq *et al*., 2011). Indigenous and locally developed sheep breeds are the result of the native ecological circumstances and joint efforts with the breeding policies of historical groups. The local livestock breeds are hardy and can survive with lower or poor quality water (i.e. brackish and muddy etc.), disease resistant and tolerant adaptation to low capacity management conditions (Raziq *et al*., 2010).

Harnai sheep is a fat tail, mutton /wool types breed. This is found in parts of Harnai, Ziarat, Loralai, Pishin, Zhob, Killa Abdullah, Killa Saifullah and Quetta districts of Balochistan. This breed is medium in size with the white compact body, head, ear tips, muffle, eyes and hooves are usually brown. Belly is somewhat pendulous. The neck and legs are white. The hock and knees may be brown spots in some animals (Khan and Isani, 1994). The average wool yield of Harnai sheep is 2.6 kg with medium fiber diameter. The breed also produces excellent quality mutton. Wool is of carpet quality and can be used for blankets and suiting. Average live weight of adult ewe and ram is 35 and 47 kg respectively (Bhutto *et al*., 1993). The population of Harnai is 0.55 million (Jehan *et al*., 2012) and the breed is scattered in a wide area. Because of the high altitude and very cold winters people of the area like its meat very much.

This breed is now under threat because of its declining number and also shrinking in size because of the last severe drought in the breed rearing area, which badly affected this breed, moreover the farmers of the area are shifting to the horticulture and plantation crop in the region. In addition indiscriminate crossbreeding with the heavy exotic "*Shenwari"* male (an *Afghani* Breed) is in practice for getting heavier lambs (Razique *et al*., 2010).

The sheep study at molecular level is very limited in Balochistan, only two studies have been reported up-till now. The first study was, on the characterization of Mengali sheep breed, by Tariq *et al*. (2012). The workers used the RAPD technique in the four indigenous sheep breeds of Balochistan. Whereas, another study was reported by Wajid *et al*. (2014) in which 11 SSR markers were amplified for the evaluation of genetic diversity of Balochi and Rakhshani sheep breeds. However, a set of (n= 16) ovine specific SSR markers were first time used for the molecular study of Harnai sheep breed of Balochistan. The findings of the current study may help in understanding the scope of genetic variability and would be helpful in making better approaches for conservation and future development in sheep breeding.

MATERIALS AND METHODS

The current study was composed on field data and laboratory work. Whole blood of (n= 50) unrelated Harnai sheep was collected from different flocks in their breeding tract (25 samples each from Multi-Purpose Sheep Research Station, Yet Abad, Lorali (MPSRF) and Sheep Research Station, (Tomagh). The animals were selected randomly from different areas. Whole blood was collected from each animal in 7ml Vacationer tubes containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The blood samples were kept cold chain and caution was taken to prevent exposing them to extreme temperatures. These blood samples were stored at –20°C until DNA isolation. The research work was conducted at Hi-Tech laboratory in the Centre for Advanced Studies in Volcanology and Biotechnology (CASVAB), University of Balochistan, Quetta.

The Harnai sheep, which is a medium size, fat tailed breed found in the northern districts of Balochistan, was analyzed by using FAO recommended microsatellites markers for the molecular characterization of the breed, DNA was extracted from (n= 50) unrelated animals (both male and females) from their breeding tract, each of 16 SSR markers were subjected to PCR using individual DNA sample for amplification.

Statistical Analysis

The obtained results were analyzed using the pop gene soft where to calculate:

1. Effective number of alleles = Ne (Kimura and Crow, 1964)
2. I = Shannon's Information index (Lewontin, 1972)
3. Observed homozygosity = Obs.Hom
4. Observed heterozygosity= Obs.Het
5. Expected homozygosity = Exp.Hom
6. Expected heterozygosity = Exp.Het
7. Gene distance = Nei
8. Average Heterozygosity = Ave. Het

RESULTS AND DISCUSSION

Amplification of alleles (PCR)

Out of 16 markers 3 did not amplify. We obtained a total number of 74 alleles ranging from 1 (OARFCB193, OARJMP29, MAF33) to 4 (OARHH47, DYMS1,

SRCRSP5). The mean number of alleles (MNA) detected for all loci was 2.448±0.869, while the average values for effective number of alleles (Ne) and Shannon's Information index (I) were found along with standard deviation to be 1.70±50.604 and 0.589±0.357 respectively.

Table 1: List of (n= 16) Ovine Specific SSR Markers

Sl. No.	Name	Primer Sequence Forward/Reverse
		F= forward R = Reverse
1	MAF65	
	F	AAAGGCCAGAGTATGCAATTAGGAG
	R	CCACTCCTCCTGAGAATATAACATG
2	OarFCB193	
	F	TTCATCTCAGACTGGGATTCAGAAAGGC
	R	GCTTGGAAATAACCCTCCTGCATCCC
3	OarJMP29	
	F	GTATACACGTGGACACCGCTTTGTAC
	R	GAAGTGGCAAGATTCAGAGGGGAAG
4	OarJMP58	
	F	GAAGTCATTGAGGGGTCGCTAACC
	R	CTTCATGTTCACAGGGTCAGGG
5	OarFCB304	
	F	CCCTAGGAGCTTTCAATAAAGAATCGG
	R	CGCTGCTGTCAACTGGGTCAGGG
6	BM8125	
	F	CTCTATCTGTGGAAAAGGTGGG
	R	GGGGGTTAGACTTCAACATACG
7	OarFCB128	
	F	ATAAAGCATCTTCTCTTTATTTCCTCGC
	R	CAGCTGAGCAACTAAGACATACATGCG
8	OarCP34	
	F	GCTGAACAATGTGATATGTTCAGG
	R	GGGACAATACTGTCTTAGATGCTGC
9	OarVH72	
	F	GGCCTCTCAAGGGGCAAGAGCAGG
	R	CTCTAGAGGATCTGGAATGCAAAGCTC
10	OarHH47	
	F	TTTATTGACAAACTCTCTTCCTAACTCCACC
	R	GTAGTTATTTAAAAAAATATCATACCTCTTAAG
11	DYMS1	
	F	AACAACATCAAACAGTAAGAG
	R	CATAGTAACAGATCTTCCTACA
12	SRCRSP1	
	F	TGCAAGAAGTTTTTCCAGAGC
	R	ACCCTGGTTTCACAAAAGG
13	SRCRSP5	
	F	GGACTCTACCAACTGAGCTACAAG
	R	GTTTCTTTGAAATGAAGCTAAAGCAATGC
14	SRCRSP9	
	F	AGAGGATTGGAAATGGAATC
	R	GCACTCTTTTCAGCCCTAATG
15	MCM140	
	F	GTTCGTACTTCTGGGTACTGGTCTC
	R	GTCCATGGATTTGCAGAGTCAG
16	MAF33	
	F	GATCTTTGTTTCAATCTATTCCAATTTC
	R	GATCATCTGAGTGTGAGTATATACAG

The means values, for observed heterozygosity (Obs. Het) expected homozygosity (Exp.Hom), expected heterozygosity (Exp.Het), and average Heterozygosity (Ave Het), were 0.598±0.299, 0.366±0.284, 0.602±0.238, 0.347±0.209 and 0.347±0.209, respectively. The value of F-statistic ranged from 0.2851 to 0.9132 for different microsatellite markers with an average of 0.515±0.021. Majority of the markers showed higher than average expected reduction in heterozygosity. The standard errors were generally low. This indicated that homozygosity prevails in the population under study. This might be due to intense inbreeding in this flock of Harnai sheep. This is the first report of microsatellite markers based characterization of Harnai sheep breed and it may contribute to the breeders for making conservation policies for the local breeds.

Banding pattern of alleles

Banding pattern of (n= 16) ovine SSR markers against DNA sample of Harnai sheep breed in Balochistan, using 5bp DNA ladder (10-100bp) on 5% agarose gel are as under:

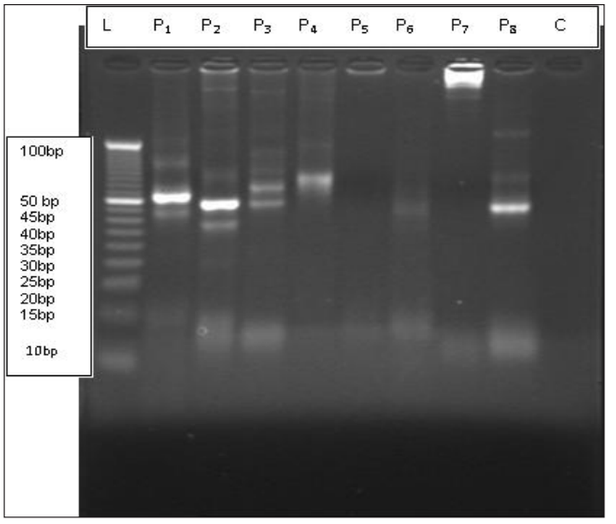

Fig. 1: Banding pattern of Primers 1-8. Primers1 (MAF65) amplified two bands (50,45 bp) suggesting two alleles; Primer 2 (OarFCB193) amplified two bands (45,43 bp) suggesting two alleles; Primer 3 (OarJMP29) amplified two bands (50, 55bp) suggesting two alleles; Primer4 (OarJMP58) amplified one band (60 bp) suggesting only one allele; Primers 5 (OarJMP58), 6 (OarFCB304) and 7 (BM8125) amplified no bands, suggesting no alleles; Primer 8 (OarFCB128) amplified three bands (50, 60 and 100bp) suggesting three alleles. L= ladder, P= primers, C= control

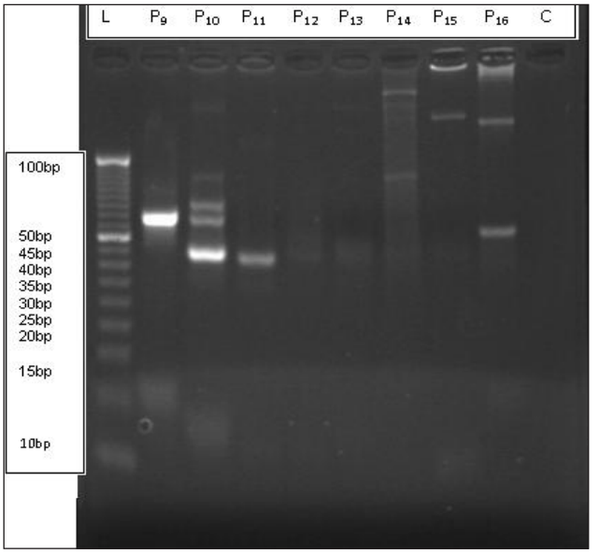

Fig 2: Banding pattern of Primers 9-16. Primer 9 (OarCP34) amplified one band (60 bp) suggesting one allele; Primer 10 (OarVH72) amplified three bands (45, 60 and 65bp) suggesting thee alleles; Primer 11 (OarHH47) amplified only one band (45 bp) suggesting one allele; Primers 12 (OarHH47) and 13 (DYMS1) showed no amplification of bands, signifying no alleles; Primer 14 (SRCRSP9) amplified two bands (90,100 bp) suggesting two alleles; Primer 15 (SRCRSP5) amplified one band (above 100 bp) suggesting only one allele; Primer 16 (MAF33) amplified two bands (50, above than 100bp) proposed two alleles. P=primers, L=ladder, C=control

Genetic diversity within Harnai sheep population

The current study shows polymorphism in the Harnai sheep population. The mean values 2.448±0.869, 1.705±0.604 and 0.589±0.357 were observed for number of alleles (Na), effective number of alleles (Ne) and Shannon's Information index (I) respectively, which are significantly less than reported by Musavi *et al.* (2011) 6.296 and 4.394 in Hazaragie sheep, Ahmed *et al*. (2014) 5.2727 and 3.9471 in Kail sheep.

The Shannon's Index (I) found during the current study was 0.589±0.357, that is less than those reported by Musavi *et al*. (2011) 1.58, Ahmed *et al*. (2014) 1.445, and Kumar *et al.* (2007) 1.419. Similarly, the average observed heterozygosity (Ho) was 0.366±0.219 found during the present study, while, Musavi *et al*. (2011), Ahmed *et al*. (2014), Kumar *et al.* (2007) and Yama *et al*. (2011) reported, 0.825, 0.512, 1.445 and 0.712 respectively.

The Mean expected heterozygosity (He) found during the present study was 0.363±0.21, which, was lesser than those reported by Musavi *et al*. (2011) 0.772, Ahmed *et al*. (2014) 0.7185 and Sharifi *et al*. (2009) 0.77, whereas, the average heterozygosity and gene diversity were found to be 0.347±0.209 and 0.347±0.209, respectively during the present study, which, were closely related to those reported by Musavi *et al*. (2011) 0.757 and 0.772.

The F-statistic values found during the current study were ranging from 0.2851 to 0.9132 for different SSR markers the average value of 0.515±0.021, low standard errors were observed during the present study, which indicates homozygosity in the Harnaisheep population under study, these observations were in close contact with Al-Barzinji *et al.* (2011) reported (0.469) inbreeding value in Hamdani sheep, however, negative (-0.069) value for inbreeding estimates were reported by Musavi *et al.* 2011. Similarly, Wajid *et al*. (2014), reported inbreeding estimates as 0.0292 and 0.0084 for Balochi and Rakhshani sheep breeds of Balochistan.

Table 2: Various Genetic Parameters of Harnai Sheep Breed

Locus	Sample Size	Na	Ne	I
MAF65	50	2.000	1.0950	0.1849
OARFCB193	50	1.000	1.000	0.000
OARJMP29	50	1.000	1.000	0.000
OARJMP58	50	3.000	1.000	0.000
OARFCB304	50	3.000	2.4694	0.9949
BM8125	50	2.000	1.6026	0.6889
OARFCB128	50	3.000	1.1980	0.3046
OARCP34	50	3.000	2.0508	0.8600
OARVH72	50	2.000	1.4578	0.6002
OARHH47	50	4.000	1.4235	0.4741
DYMS1	50	4.000	3.5072	1.3011
SRCRSP1	50	2.000	1.7664	0.6255
SRCRSP5	50	4.000	2.8471	1.1673
SRCRSP9	50	3.000	1.9055	0.8158
MCM140	50	3.000	2.6022	1.0209
MAF33	50	1.000	1.000	0.000

Ne = Effective number of alleles [Kimura and Crow (1964)].

I = Shannon's Information index [Lewontin (1972)].

The Table 3 indicates to the average values along with their standard deviations to be 0.598±0.299, 0.366±0.284, 0.602±0.238, 0.363±0.219, 0.347±0.209 and 0.347±0.209 for observed heterozygosity (Obs.Het) expected homozygosity (Exp.Hom), expected heterozygosity (Exp.Het), effective number of allele (Ne) average Heterozygosity (Ave Het), respectively**.** The lowest observed homozygosity was 0.363 (OARAE129, HUJ616) and the highest was 1.00 (OARFCB193, OARJMP29, QARJMP58, OARHH47 and ILSTS 28), similarly the highest observed heterozygosity was calculated as 0. 818 (SRCRSP5 and MCM140) while the highest value for expected heterozygosity was 0.748 (DYMSI).

Fixation indices or F-statistic gives statistically expected amount of heterozygosity in a population or degree of reduction in heterozygosity (Table 4). The value of F-statistic ranged from 0.2851 to 0.9132 for different microsatellite markers with an average of 0.515±0.021. Majority of the markers showed higher than average expected reduction in heterozygosity. The standard errors were generally low. This indicated that homozygosity prevails in the population under study. This might be due to intense inbreeding in this flock of Harnai sheep.

Table 3: Some Genetic Diversity Parameters of Harnai Sheep Breed

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het	Nei	Ave_Het
MAF65	50	0.9091	0.0909	0.9091	0.0909	0.0868	0.0868
OARFCB193	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000
OARJMP29	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000
OARJMP58	50	1.0000	0.0000	1.0000	0.0000	0.0000	0.0000
OARFCB304	50	0.4545	0.5455	0.3766	0.6234	0.595	0.595
BM8125	50	0.5455	0.4545	0.6061	0.3939	0.376	0.376
OARFCB128	50	0.8182	0.1818	0.8268	0.1732	0.1653	0.1653
OARCP34	50	0.2727	0.7273	0.4632	0.536	0.5124	0.5124
OARVH72	50	0.6364	0.3636	0.671	0.329	0.314	0.314
OARHH47	50	1.0000	0.0000	0.6883	0.312	0.2975	0.2975
DYMS1	50	0.3636	0.6364	0.2511	0.749	0.7149	0.7149
SRCRSP1	50	0.3636	0.6364	0.5455	0.455	0.4339	0.4339
SRCRSP5	50	0.1818	0.8182	0.3203	0.679	0.6488	0.6488
SRCRSP9	50	0.5455	0.4545	0.5022	0.498	0.4752	0.4752
MCM140	50	0.1818	0.8182	0.355	0.645	0.6157	0.6157
MAF33	50	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Mean	50	0.598	0.366	0.602	0.363	0.347	0.347
S.Dev.		0.299	0.284	0.238	0.219	0.209	0.209

Obs.Hom = observed homozygosity, Obs.Het = observed heterozygosity, Exp.Hom = expected homozygosity, Exp.Het = expected heterozygosity, Ne = effective number of allele, Nei = gene distance and Ave. Het= average Heterozygosity**.**

Table 4: Summary of F- Statistics of Harnai Sheep Population

Locus	N	k	Obs F	Min F	Max F	Mean	SE	L95	U95
MAF65	50	2	0.9132	0.5000	0.9132	0.7414	0.0224	0.5000	0.9132
OARFCB193	50	1	0.0000	0.0000	0.0000	****	****	****	****
OARJMP29	50	1	0.0000	0.0000	0.0000	****	****	****	****
OARJMP58	50	1	0.0000	0.0000	0.0000	****	****	****	****
OARFCB304	50	3	0.4050	0.3333	0.8347	0.5697	0.0201	0.3512	0.8306
BM8125	50	3	0.6240	0.3333	0.8347	0.5673	0.0206	0.3512	0.8306
OARFCB128	50	2	0.8347	0.5000	0.9132	0.7381	0.0232	0.5000	0.9132
OARCP34	50	3	0.4876	0.3333	0.8347	0.5670	0.0210	0.3430	0.8306
OARVH72	50	3	0.6860	0.3333	0.8347	0.5666	0.0211	0.3430	0.8306
OARHH47	50	2	0.7025	0.5000	0.9132	0.7356	0.0218	0.5000	0.9132
DYMS1	50	4	0.2851	0.2500	0.7645	0.4494	0.0148	0.2769	0.7521
SRCRSP1	50	3	0.5661	0.5000	0.9132	0.7353	0.0221	0.5000	0.9132
SRCRSP5	50	4	0.3512	0.2500	0.7645	0.4515	0.0157	0.2769	0.7521
SRCRSP9	50	3	0.5248	0.3333	0.8347	0.5644	0.0203	0.3512	0.8306
MCM140	50	3	0.3843	0.3333	0.8347	0.5606	0.0200	0.3512	0.8306
MAF33	50	1	0.0000	0.0000	0.0000	****	****	****	****
Mean			0.515	0.342	0.742	0.627	0.021	0.408	0.857
S.Dev.			0.261	0.166	0.306	0.106	0.002	0.087	0.056

CONCLUSION

It can be concluded from the results of the current study that Harnai sheep is a distinct breed from the other sheep breeds of Balochistan, having molecular level variation among the sample of the population. The breed characterization is the first step towards the conservation of this local asset.

ACKNOWLEDGEMENTS

We are thankful to the Livestock and Dairy Development Department, Balochistan and Arid Zone of Pakistan Agriculture Research Council (PARC), Quetta for their support in data collection and other facilities.

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