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Full Length Research Paper

Genetic variations between indigenous fat-tailed sheep populations

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Blood samples were collected from a total 816 sheep of both sexes in three Iranian fat-tailed breeds (Sangsari, Makoei, indigenous sheep on firoozkouh mountain) serum, plasma and erythrocyte were separated and were frozen at -20°C. Variation in their blood proteins, albumin, haemoglobin and transferrin were examined to characterize the breeds and to obtain genetic relationship among them. Only transferrin was polymorphic in all breeds investigated; while albumin was monomorphic for S allele and haemoglobin was fixed for the B allele in three breeds.

Keywords: Sangsari, makoei, firoozkouhi, fat-tailed, albumin, transferrin, haemoglobin.

INTRODUCTION

Sheep are an important source of meat in Iran. These indigenous sheep form a very valuable genetics resource for sustainable utilization of arid and semi-arid lands which form approximately more than 90% of total land mass of the country. There is evidence that they are resistant to many endemic diseases in Iran. Despite the importance of indigenous sheep in Iran, breeds information is scarce on their genetic make-up. Artificial selection has resulted in tremendous change and wide diversity in phenotypic (morphological, physiological and behavioral) characters of domesticated animal, result in a variety of genotype with specific production properties and adaptation capability. Blood group and protein have been used widely to characterize genetic diversity (Deza et al., 2000; Missohou et al., 1998; Ndamunkong, 1995), while some blood proteins have been associated with quantitative and adaptability trait (Dally et al., 1980; Missouhou et al., 1998; Pierragostini et al., 1994). This study was designed to evaluate the levels of blood protein polymorphism in fat-tailed sheep in Iran.

MATERIALS AND METHODS

Blood was obtain from 816 sheep in the three Iranian sheep breeds,Sangsari, native Firoozkouhi sheep and Makoei, from which 576, 120 and 120 animals were used, respectively. Blood sampling was performed at random in different region, those breeds raised. For minimizing the probability of any close genetics relationships, farmers were interviewed in detail on pedigree of animal before sampling. Blood samples were centrifuged for 25 min at 1850 g, then plasma, Buffy coat and red blood cells were removed separately by suction into 2 ml micro tubes that were stored at -20°C. Three protein coding loci albumin (Al), transferrin (Tf) and haemoglobin were analyzed. The electrophoresis assay and staining protocols used are shown in Table 1. Gene frequency for polymorphic were computed by the gene counting method for Tf, Hb and Al loci. Gene homogeneity index (H.I.) for quantifying the genetic homogeneity of population was calculated using the following formula $n/n - 1[(p_1^2 + p_2^2 + \ldots + p_n^2) - 1/n]$, where n is the number of alleles and p_n is the frequency of the n-th alleles at a gene locus. Genetics variability was quantified in term of the proportion of polymorphic loci(H), calculated using the following formulae: P_{poly} = number of polymorphic loci/ total number of loci examined, (polymorphic locus being defined as the frequency of the most common allele at locus of less than 0.99) and average heterozygosity (H) = 1 - $\sum p_i 2$, (p_i = frequency of i-th allele). Nei (1972) standard genetic distance (D_s between was calculated as a measure of genetic similarity between population. Carvalli-sforza and Edwards (1967) chord genetic distance (D_c) was used for phylogenetic reconstructions using unweighted pair-group method (UPGMA) (Figure 1).

RESULTS

Gene frequency for transferrin, albumin and haemoglobin were shown (Table 2). Transferrin just was polymorphic

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Table 1. Separation method of blood proteins used for the study.

Item	Haemoglobin	Albumin	Transferrin
Alleles	A,B	F,S	
System	STAGE[b]	STAGE[b]	PAGE[a]
Electrode buffer	Tris-borate-EDTA, pH 8.7	Borate-sodium hydroxide, pH 8.7	Tris-citrate
Gel buffer	Tris-borate-EDTA pH 8.6	Tris-citrate, pH 6.2	Tris-borate
Stain	Nigrosin	Nigrosin	Coomasie BlueR250
Source	Tucker and Clarke(1980)	Tucker and Clarke(1980)	Tucker and Clarke(1980)

[a] Polyacrylamide gel electrophoresis. [b] Starch gel electrophoresis.

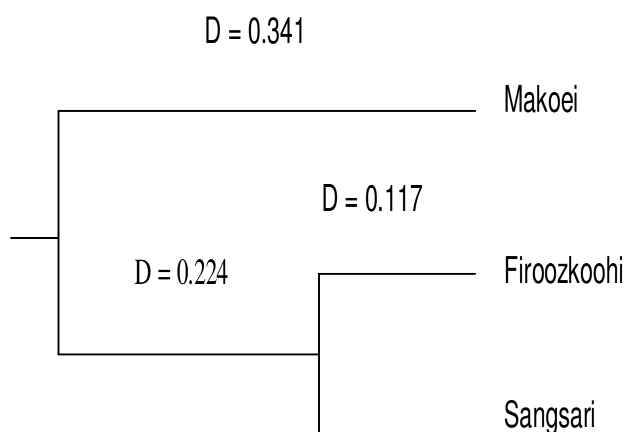

Figure 1. Cluster analysis using UPGMA tree with Dc distance matrix.

locus, in which those alleles were observed are A, B, C, D, E, G, K, L, M, Q, I and P that allele C in Sangsari and allele B in Firoozkouhi and Makoei are most frequent alleles. A, B, C, D, G alleles were present in all breeds. The allele P in Makoei, I in Firoozkouhi and Q in Sangsari was found only. The allele's frequencies in detail are shown in Table 2. Albumin and haemoglobin was monomorphic for AlS and HbS allele in all three fat-tailed sheep population. Measures of genetics variability for each population are given in Table 3. Expected heterozygosity estimates within breeds at the blood protein loci analysed (Table 3), showed that the Makoei had the largest (0.261), whereas the two other breeds exhibited the approximately equal heterozygosity. The magnitude the genetic distances were estimated by Nei's method that was shown in Table 4. The distance between three breeds was not considerable. The largest one was between Firoozkouhi and Makoei (0.007).

DISCUSSION

Cryptic polymorphisms of blood groups or proteins that, have not been deliberately selected by man, shows simple patterns of evolutionary change. For this reason the use of allozyme polymorphism can give accurate estimate of relationship between populations (Ordas and San Primitivo, 1986). Maintaining genetics variance within a pure breeding population is complex problem. In this study allelic constitutions observed were generally similar in the studied populations. Predominance alleles of three blood protein, Als, HbB and TfC agree with results obtained on fat-tailed breed and Namaqua sheep (Clarke et al., 1989). Many researches have been shown that all alleles observed in these studied breeds were not observed completely on other exotic breeds especially in Merino breed which doesn't have fat-tailed. This result indicates that, the indigenous breeds are markedly divergent from exotic breeds. Fixation of certain alleles and loss of others within the fat-tailed sheep are indigenous of evolutionary change either as a result of natural or random genetic drift or may be that the genes of these blood proteins have been linked with genes that affect economic important traits. In addition, these proteins may have direct effect on economic important traits in which selection based on those traits indirectly change allele frequency. The degree of within and between breed genetic diversity which was very low at the level of genetic variation have also been reported in other breeds of sheep studied using blood proteins polymorphism (Clarke et al.,1989; Nguyen et al., 1992). This low level of genetic variation may be attributed to the low polymorphism and variability of protein markers. Nei genetic distance obtained in this study was within the range of 0.001 - 0.007 as indicated by Nei (1976) in these Iranian local breeds. Similarly, Ordas and San Primitivo (1986), estimated genetic distances between Spanish dairy sheep breeds in the range of 0.0094 - 0.055 using data from eight genetic system. Zanotti et al. (1990) used data of four blood groups and six protein coding loci and found a distance equal to 0.012 - 0.060 (mean = 0.039) between five Italian sheep breeds. However, micro-satellite markers have provided much more accurate estimate of genetics distances in sheep (Arranz et al., 1998; Diez-Tascon et al., 2000). The genetic distance estimates indicated a close genetic relationship between these studied fat-tailed sheep populations (Sangsari, Makoei, indigenous sheep on Firoozkouh mountain and Kenya indigenous sheep) and this may have been the

Table 2. Gene frequencies for each population studied.

Population analyzed			Alleles	Locus
Makoei	Firoozkouhi	Sangsari		
-	-	-	F	Al
1	1	1	S	Al
-	-	-	A	Hb
1	1	1	B	Hb
0.250	0.189	0.155	A	Tf
0.319	0.379	0.248	B	Tf
0.263	0.241	0.297	C	Tf
0.111	0.103	0.209	D	Tf
-	0.034	0.030	E	Tf
0.013	0.017	0.035	G	Tf
-	-	0.010	K	Tf
-	0.017	0.008	L	Tf
0.013	-	0.002	M	Tf
-	-	0.002	Q	Tf
-	0.017	-	I	Tf
0.027	-	-	P	Tf

Table 3. Genetic variability measures for each population analyzed.

Population	Mean expected heterozygosity	Mean observed heterozigosity	Percentage of polymorphic loci (0.95 criterion)	Mean effective number alleles per locus
Sangsari	0.251 ± 0.436	0.259 ± 0.449	33.33	2.012 ± 1.753
Firoozkouhi	0.250 ± 0.434	0.229 ± 0.398	33.33	1.995 ± 1.723
Makoei	0.260 ± 0.450	0.257 ± 0.446	33.33	2.180 ± 2.044

Table 4. Measures of genetic similarities.

Population	Sangsari	Firoozkouhi	Makoei
Makoei	0.994	0.993	-
Firoozkouhi	0.998	-	0.007
Sangsari	-	0.001	0.005

Genetic identity (above diagonal) and genetic distance (below diagonal).

result of admixture between populations due to the historical migrations of populations with their livestock and the widespread exchange of genetic material through trade, dower payment (Mwacharo, 2000).

The range of mean expected heterozygosity by using blood protein markers in the three studied breeds was between 0.25 and 0.26. Takezaki and Nei (1996) showed that for markers to be useful for measuring genetic variation, they should have an average heterozygosity between 0.3 and 0.8 in the population. Estimates of mean expected heterozygosity obtained in this study neither were in this range of those obtained for sheep breeds using blood protein markers (Nguyen et al., 1992; Ibeagha-Awemu and Erhardt, 2004) nor in the result range. The phylogenetic tree constructed separates the Makoei from two other indigenous fat-tailed sheep .Close genetic relationship observed between Sangsari and Firoozkouhi is not easy to explain as these two populations occupy different ecological zone. However this closeness may be resulting of migration and interbreeding between them. Finally, each of these breeds has its importance because of its exceptional reproduction or production characteristics, its adaptation to regional environment or its use for the genetic improvement of other breeds.

Conclusion

The aim of study was provide information about three blood protein allele’s frequency and utilization this distance for improving breeding program. However, this study only analyzed large sample size of total population of sheep in

Iran but number of loci which genotyped was small. Thus, it is recommended that this study may be continued to more loci. The use of DNA microsatellite marker is highly recommended.

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