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Full Length Research Paper

Genetic diversity of Najdi sheep based on microsatellite analysis

Muneeb M. Musthafa[1,2]*, R. S. Aljummah[2] and M. A. Alshaik[2]

[1]National Institute of Animal Science and Veterinary Medicine, Chinese Academy of Agricultural Sciences, 2 W, Yuanmingyuan Road, Beijing 100193, People Republic of China.
[2]Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Kingdom of Saudi Arabia.

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The prime objective of this research was to measure the genetic polymorphism of main sheep breed of Saudi Arabia, Najdi. Randomly selected 49 blood samples were used to extract the DNA followed by polymerase chain reaction (PCR), using 19 microsatellite markers, which were used to investigate the genetic differentiation. Altogether, 173 alleles were identified ranging from 2 to 14, with the mean observed number alleles per locus of 9.11 ± 3.54. Apart from that, eight loci showed breed specific alleles which is critical in terms of conservation. The observed heterozygosity, expected heterozygosity, polymorphic information content and Shannon index, were 0.67 ± 0.19, 0.75 ± 0.14, 0.71 ± 0.16 and 1.69 ± 0.51, respectively. Therefore, considerable amount of genetic polymorphism has been shown by Najdi. Inbreeding coefficient of 0.13 exhibited moderate level of inbreeding prevailing, which may be partly due to the Wahlund effect (sub-population structure) at level of sampling. Nine out of the 19 loci encountered significant departure from Hardy Weinberg Equilibrium ($p < 0.05$). Based on the bottleneck analysis, there was no bottleneck effect in Najdi. This paper reports a comprehensive study on genetic diversity of Najdi, hence, it would be used for further advancement of this breed towards utilizing them sustainably.

Key words: Polymerase chain reaction (PCR), diversity, microsatellites, sheep, inbreeding.

INTRODUCTION

Sheep is one of the earliest ruminants to be domesticated by human at Fertile Crescent, 9000 years ago (Peter et al., 2007; Tapio et al., 2006; Zeder et al., 2006), originating from at least three ancestral subspecies of the wild Mouflon, known as primitive type (Chessa et al., 2009; Pedrosa et al., 2005). It has been estimated that more than 850 commercial and domestic sheep breeds are reported all over the world (Rege and Gibson, 2003).

Studies on genetic diversity of small ruminants have been extravagantly accelerated over the past decades based on microsatellite markers (Bhatia and Arora, 2005). It has been proven to be useful for genetic diversity studies, parentage test, linkage analysis and population genetic studies, due to their superior features over the other markers (Bruford and Wayne, 1993). These advantages led the way for using microsatellites to measure genetic diversity among animals like cattle (Egito et al., 2007; MacHugh et al., 1997), sheep (Arora et al., 2011; Gornas et al., 2011; Kusza et al., 2010), goat (Dixit et al., 2008; Mahmoudi et al., 2010; Serrano et al., 2009), camel (Ahmed et al., 2010; Mehta and Sahani, 2007; Schulz et al., 2010), buffaloes (Moiolo et al., 2001; Arora et al., 2004; El-Kholy et al., 2006) and Arabian Oryx (Arif et al., 2010; Khan et al., 2011). Evaluation of genetic diversity is the foremost step towards conservation and sustainable utilization of genetic resources

*Corresponding author. E-mail: muneemmm@gmail.com.

Abbreviations: PIC, Polymorphic information content; **PCR,** polymerase chain reaction; **HWE,** Hardy Weinberg equilibrium.

(Dalvit et al., 2008; Glowatzki-Mullis et al., 2008; Kevorkian et al., 2010), and could prove to be a handful tool to maintain the breeds.

Sheep play an important role in the livelihood of indigenous people and nomads in Saudi Arabia, and the native sheep breeds are distributed all over the country (Pritchard et al., 1977). Najdi is a well adapted multi-purpose breed, primarily used for meat, milk and wool production. Najdi has some unique features such as black hair coat with white head, convex head profile and large, pendulous ears (Pritchart et al., 1977), long legs and fat tailed with coarse fleece (Ali and Al-Noami, 1992). Body weights of mature ewes average around 50 kg, while rams are 5 to 10 kg heavier (Pritchart et al., 1977). Even though Najdi plays a variety of roles in a farming community, seldom studies have been undertaken regarding genotypic variability of Najdi sheep population found in Saudi Arabia. In the study carried out by Peter et al. (2007) on genetic diversity and subdivision of 57 European and Middle Eastern sheep breeds, Najdi was also included with 31 samples from Saudi Arabia but it was not purely about Najdi. Therefore, the main focus of this research was to unravel the genetic diversity of Najdi sheep using 19 microsatellite markers in an extended manner.

MATERIALS AND METHODS

Sampling

Random blood samples were collected from 49 typical Najdi sheep found in different farms of central region of Saudi Arabia. Jugular vein derived 10 ml blood samples under aseptic conditions using ethylenediaminetetraacetic acid (EDTA) anticoagulant were brought to the laboratory on ice box for further analysis. DNA extraction was carried out using GFX™ genomic blood DNA purification Kit (Amersham Pharmacia Biotech, USA).

Polymerase chain reaction (PCR) amplification

19 International Society for Animal Genetics (ISAG) recommended fluorescent labeled polymorphic microsatellite markers (Table 1) found in 15 different chromosomes were used to amplify the extracted DNA. Only the forward primer of the each primer pair was labeled with the four of the following fluorescent dyes: FAM-Blue, PET-Red, NED-Yellow and VIC-Green provided by Applied Biosystems™ (CA, USA). The PCR amplification was performed using a standard procedure by Applied Biosystems™ GeneAmp® PCR system 9700 (CA, USA) with PCR mix volume of 10 µl. Amplified products were analyzed by ABI PRISM genetic analyzer 3130 (Applied Biosystems™, CA, USA) following manufacturer's protocol. Microsatellite fragment sizing was performed by the GeneMapper® version 4.0 (Applied Biosystems™, CA, USA) and the size standard peaks were defined by the user. Allele calling was performed with the software and checked manually to avoid any false calling.

Statistical analysis

Statistical analysis was carried out using Cervus (Kalinowski et al., 2007) version 3.0.3 from Field Genetics Limited to find out the expected heterozygosity (H_e), observed heterozygosity (H_o) and polymorphic information content (PIC). Wright's F-statistics was used to calculate F_{is} by GenePop version 4.0.10 (Raymond and Rousset, 1995). The exact test for deviations from Hardy-Weinberg equilibrium (HWE) also calculated using Genepop, while, Bottleneck analysis was carried out using Bottleneck version 1.2.02 (Cornuet and Luikart, 1996). Furthermore, Popgene version 1.31 (Yeh et al., 1999) was used to calculate the effective number of alleles, PIC and Shannon weaver diversity index (I) and Ewens-Watterson test for neutrality of the microsatellite markers.

RESULTS

A total of 173 alleles were found across 19 investigated loci and all markers were found to be polymorphic in Najdi population. ILSTS044 and OARFCB226 showed the highest number of alleles per locus (14) while MAF214 showed the lowest (2), with the mean number of 9.11 ± 3.54. Considerable level of genetic variability was observed in terms of number of alleles observed in all tested loci (>2) (Crawford et al., 1995). The mean expected heterozygosity was 0.75 whereas, the mean observed heterozygosity was 0.67 (Table 2). Five of the 19 loci showed higher observed heterozygosity than expected. Eight of the loci explicated breed specific alleles (Table 3), with 17 alleles out of total 173. The Mean PIC and mean Shannon index were 0.71 ± 0.16 and 1.69 ± 0.51, respectively (Table 2). Mean inbreeding coefficient (F_{is}) values by Weir and Cockerham method, and Robertson and Hill method showed 0.13. Based on Weir and Cockerham approach, four loci showed negative inbreeding values, whereas only two of loci showed negative values by Robertson and Hill approach. 10 loci corresponded to HWE ($p < 0.05$) (Table 4). Ewens-Watterson test for neutrality of microsatellite markers showed that none of the tested loci were under selection (Table 5), except OarFCB20, the rest of the loci were within the upper and lower 95% confidence interval. Bottleneck analysis was conducted to assess the bottleneck effect in Najdi, and the results show no bottleneck in recent past (Table 6). The quantitative measure of genetic bottleneck was tested using the mode shift indicator method and it displayed a normal 'L' shaped curve, confirming there is no bottleneck in Najdi in recent past (Figure 1).

DISCUSSION

Arguably, there is very few information available on the genetic diversity of Najdi. Allele frequency estimates are crucial in measuring the polymorphism hence; the estimates of polymorphism highly depend on number of alleles and allele frequencies (Cervini et al., 2006). The observed number of alleles at each locus is an indication of genetic diversity at those loci and having a direct effect on within breed variability (Buchanan et al., 1994; Saitbekova et al., 1999). The allele variability measure

Table 1. Primers, sequence, annealing temperature, chromosome number, and their sizes.

Marker	Sequences (5′ to 3′)	Annealing temperature (ºC)	Chromosomal number	Size (bp)
HUJ616	F-TTCAAACTACACATTGACAGGG F-TTCAAACTACACATTGACAGGG	55	13	118-144
BM1329	F-TGTTTTGATGGAACACAGCC R-TGGATTTAGACCAGGGTTGG	55	3	135-185
OarFCB11	F-GTTAGTACAAGGATGACAAGAGGCAC R-GACTCTAGAGGATCGCAAAGAACCAG	58	2	121-143
OARFCB20	F- GGAAAACCCCCATATATACCTATAC R-AAATGTGTTTAAGATTCCATACATGTG	58	2	93-112
SRCRSP9	F- CGGGGATCTGTTCTATGAAC R- TGATTAGCTGGCTGAATGTCC	55	10	95-135
MAF214	AATGCAGGAGATCTGAGGCAGGGACG GGGTGATCTTAGGGAGGTTTTGGAGG	60	16	174-282
MAF209	F-CACGGAGTCACAAAGAGTCAGACC R- GCAGGACTCTACGGGGCCTTTGC	65	4	100-127
OARFCB226	F-CTATATGTTGCCTTTCCCTTCCTGC R-GTGAGTCCCATAGAGCATAAGCTC	56	7	110-160
HSC	F-CTGCCAATGCAGAGACACAAGA R-GTCTGTCTCCTGTCTTGTCATC	56	20	263-297
ILSTS005	F-GGAAGCAATTGAAATCTATAGCC R-TGTTCTGTGAGTTTGTAAGC	55	10	181-216
OARHH47	F-TTTATTGACAAACTCTCTTCCTAACTCCACC R-GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	56	18	130-152
MCM42	CATCTTTCAAAAGAACTCCGAAAGTG CTTGGAATCCTTCCTAACTTTCGG	55	9	86-109
OARVH72	F-CTCTAGAGGATCTGGAATGCAAAGCTC R-GGCCTCTCAAGGGGCAAGAGCAGG	56	25	121-147
DYMS1	F-AACAACATCAAACAGTAAGAG R-CATAGTAACAGATCTTCCTACA	58	23	145-210
ILSTS044	F-AGT CAC CCAAAAGTAACTGG R-ACATGTTGTATTCCAAGTGC	55	Ann	145-177
OARJMP29	F-GTATACACGTGGACACCGCTTTGTAC R-GAAGTGGCAAGATTCAGAGGGGAAG	55	24	96-150
BM8125	F-CTCTATCTGTGGAAAAGGTGGG R-GGGGGTTAGACTTCAACATACG	55	17	116-122

Table 1. Contd.

SRCRSP5	F-TGAAATGAAGCTAAAGCAATGC R-GGACTCTACCAACTGAGCTACAAG	56	12	110-170
TGLA53	F-GCTTTCAGAAATAGTTTGCATTCA R-ATCTTCACATGATATTACAGCAGA	55	16	142-166

Ann, Anonymous.

Table 2. Variability parameters of Najdi sheep.

Locus name	n_a	H_o	H_e	PIC value	Shannon
MCM42	6	0.67	0.66	0.60	1.27
OarFCB20	13	0.92	0.89	0.87	2.28
OARVH72	9	0.65	0.73	0.68	1.57
TGLA53	10	0.77	0.84	0.81	1.99
DYMS1	12	0.67	0.88	0.86	2.19
ILSTS044	14	0.77	0.87	0.84	2.22
ILSTS05	9	0.69	0.79	0.75	1.75
MAF209	9	0.67	0.82	0.78	1.84
BM8125	6	0.56	0.60	0.55	1.16
MAF214	2	0.23	0.46	0.35	0.64
OARFCB11	10	0.81	0.86	0.83	2.04
OARJMP29	10	0.67	0.79	0.75	1.75
HUJ616	7	0.67	0.67	0.61	1.28
OarFCB226	14	0.81	0.86	0.84	2.22
SRCRSP09	5	0.77	0.73	0.68	1.40
BM1329	9	0.25	0.49	0.47	1.16
HSC	13	0.92	0.88	0.86	2.23
OARHH47	12	0.81	0.88	0.85	2.21
SRCRSP5	3	0.42	0.58	0.51	0.97
Mean	9.11	0.67	0.75	0.71	1.69
SD	3.54	0.19	0.14	0.16	0.51

(n_a, Number of observed alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, polymorphic information content.

(9.105) demonstrated considerable amount of genetic diversity in Najdi sheep, whereas study by Peter et al. (2007) displayed allelic richness of Najdi sheep as 7.10. This study can be compared with some of the Indian sheep breeds such as Ganjam breed (5.48), Chokla (5.32), Medras Red Sheep (5.00), Garole (6.20), Muzaffarnagri (5.04), Jalauni (5.92), Kheri (5.30), Nali (5.52), Vembur sheep (5.88) and Shahabadi (5.56) (Arora et al., 2010; Sodhi et al., 2006; Prema et al., 2008; Sodhi et al., 2003; Arora and Bhatia, 2004; Arora et al., 2008; Bhatia et al., 2005; Sodhi et al., 2006; Pramod et al., 2009; Pandey et al., 2010), respectively and they showed lower mean observed number of alleles. Pakistani sheep breeds (3.80) (Ibrahim et al., 2010) and Iranian sheep breeds (6.48) (Seidani et al., 2009) showed lower allelic variability.

Alpine sheep breeds (19.00) (Dalvit et al., 2008), Egyptian sheep breeds (10.30), (El Nahas et al., 2008), Spanish sheep breeds (13.30) (Calvo et al., 2011), Chilean sheep breeds (18.33) (Barra et al., 2010), Bhutan sheep breeds (13.38) (Dorji et al., 2010), Albanian local breeds (16.00) (Hoda et al., 2009), European sheep breeds (19.90) (Handley et al., 2007) and Gentile di Puglia sheep of Italy (9.68) (d'Angelo et al., 2009) showed higher allele diversity when compared to Najdi sheep. Currently, there is an increasing attention about the preservation of private alleles found in domestic animals, since they are unique to particular breed (Kusza

Table 3. Breed specific alleles.

Locus	Length	Frequency	Total percentage
ILSTS044	179	0.04	29.1
	181	0.01	
	183	0.08	
	185	0.14	
	187	0.02	
MAF209	128	0.05	11.4
	130	0.06	
BM8125	108	0.01	64.5
	110	0.05	
	114	0.58	
OARFCB11	145	0.16	17.7
	147	0.02	
	153	0.58	
SRCRSP9	155	0.22	80.2
OARJMP29	155	0.01	1
HUS616	157	0.01	1
HSC	260	0.01	1

Table 4. Inbreeding coefficient values (F_{is}) and Hardy-Weinberg equilibrium probability values.

Locus name	F_{is} value		HWE
	W and C	R and H	
MCM42	-0.0033	0.0447	0.3849
OarFCB20	-0.0266	-0.0071	0.9242
OARVH72	0.1124	0.1805	0.0000
TGLA53	0.0779	0.0557	0.8546
DYMS1	0.2423	0.1715	0.0001
ILSTS044	0.1112	0.0878	0.0149
ILSTS05	0.1255	0.1871	0.0000
MAF209	0.1857	0.1659	0.0066
BM8125	0.0652	0.0597	0.1595
MAF214	0.5000	0.5079	0.0005
OarFCB11	0.0569	0.0446	0.5540
OARJMP29	0.1546	0.0656	0.9535
HUJ616	0.0027	0.0023	0.9692
OarFCB226	0.0612	0.0827	0.0000
SRCRSP09	-0.0543	0.0282	0.0081
BM1329	0.4905	0.4763	0.0000
HSC	-0.0418	-0.0275	0.1377
OARHH47	0.0731	0.0555	0.0411
SRCRSP5	0.2822	0.2364	0.394
Mean	0.127126	0.127253	

,W and C, Weir and Cockerham method; R and H, Robertson and Hill method; HWE, Hardy Weinberg Equilibrium.

Table 5. The Ewens-Watterson test for neutrality.

Locus name	Observed F	SE	L95	U95
MCM42	0.3424	0.0197	0.2270	0.7706
OarFCB20	0.1161	0.0045	0.1204	0.3743
OARVH72	0.2808	0.0101	0.1656	0.5499
TGLA53	0.1734	0.0089	0.1500	0.5033
DYMS1	0.1315	0.0060	0.1311	0.4332
ILSTS044	0.1428	0.0034	0.1155	0.3372
ILSTS05	0.2231	0.0102	0.1656	0.5456
MAF209	0.1914	0.0091	0.1667	0.5326
BM8125	0.4049	0.0209	0.2307	0.7700
MAF214	0.5488	0.0287	0.5009	0.9794
OarFCB11	0.1480	0.0090	0.1510	0.5295
OARJMP29	0.2209	0.0090	0.1493	0.5080
HUJ616	0.3385	0.0170	0.2072	0.7166
OarFCB226	0.1441	0.0035	0.1141	0.3424
SRCRSP09	0.2760	0.0242	0.2663	0.8422
BM1329	0.5169	0.0102	0.1701	0.5603
HSC	0.1289	0.0045	0.1189	0.3678
OARHH47	0.1332	0.0052	0.1278	0.4171
SRCRSP5	0.4273	0.0320	0.3752	0.9590

(Observed F- Observed frequency, SE- standard error, L95- Lower 95%, U95- Upper 95%).

Table 6. Bottleneck analysis of Najdi sheep.

Test	Models of microsatellite evolutions		
	IAM	TPM	SMM
Sign test			
Expected number of loci with heterozygosity excess	11.1600	11.1100	11.0000
Observed number of loci with heterozygosity deficiency	1	5	12
Probability	0.0006	0.1308	0.0524
Standardized differences test			
T_2 values	3.3510	0.8330	-4.1780
Probability	0.0004	0.2025	0.0001
Wilcoxon rank test			
Probability (one tail for heterozygosity deficiency)	0.9998	0.9753	0.0247
Probability (one tail for heterozygosity excess)	0.0006	0.0273	0.9778
Probability (two tail for heterozygosity deficiency and excess)	0.0012	0.0546	0.0494

IAM, infinite allele model; TPM, two phase model; SMM, stepwise mutation model.

et al., 2010). Out of the eight loci which showed 10% private alleles, five of them were with higher frequencies, therefore it can be considered as a measure of genetic distinctiveness of these loci in Najdi.

The mean gene diversity of Najdi sheep (0.75) showed very close value for gene diversity (0.76) showed by the research carried out by Peter et al. (2007) on Najdi sheep while the observed heterozygosity was marginally higher (0.70) than this study (0.67). Swiss sheep breeds (0.75), Canadian sheep breeds (0.74) (Farid et al., 2000), Gentile di Puglia sheep of Italy (0.767) (d'Angelo et al., 2009), Sicilian sheep breeds (Tolone et al., 2012), Sanjabi sheep breed of Iran (Solimani et al., 2011) and Albanian sheep breeds (0.77) (Hoda et al., 2009) showed close values to Najdi. On the other hand, Alpine sheep breeds (0.82) (Dalvit et al., 2008), Iranian sheep (0.77)

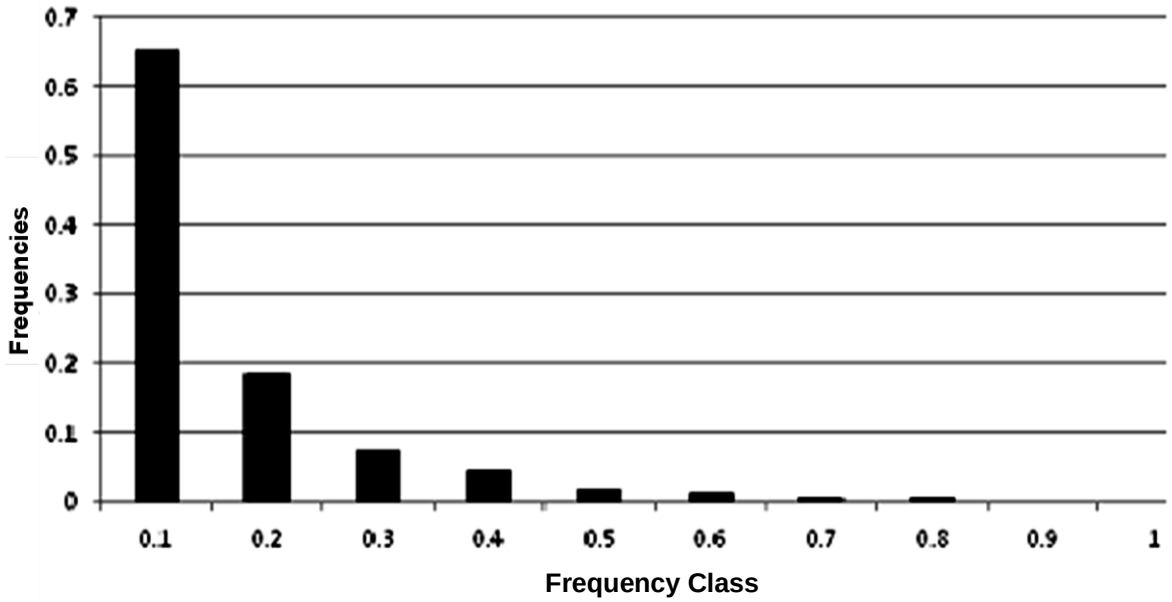

Figure 1. Mode shift analysis depicting absence of genetic bottleneck in Najdi sheep. Normal 'L' shape curve depicting that, there is no bottleneck in Najdi.

(Seidani et al., 2009) three of the Egyptian sheep breeds (El Nahas et al., 2008), Chilean sheep breeds (0.81) (Barra et al., 2010) and Pelt sheep (0.81) (Nanekarani et al., 2010) showed higher gene diversity values when compared to Najdi sheep. Based on the heterozygosity measurements, Najdi breed.

PIC is a measure of the informativeness of the marker and it ranges from 0 to 1. Loci with PIC value of 1 or close to 1 with many numbers of alleles are normally desired for genetic diversity studies (Botstein et al., 1980). PIC of the markers used in this study was quite high with the mean of 0.71. Two of the markers showed PIC values lower than 0.5 (MAF214 and BM1329), implying moderately informative (0.5 > PIC > 0.25); the rest of them were highly informative (PIC > 0.5). Nevertheless, these markers are extensively used in sheep genetic diversity studies throughout. Shannon index also showed the mean value (1.69), reflecting the species richness is health. Ewen-Watterson test (Manly, 1985) for neutrality of markers showed none of the tested markers favor any kind of selection. OarFCB20 narrowly below the lower 95% cut off and the other 18 markers used in this study exhibited the observed F (sum of square of allele frequency) within the upper and lower 95% confidence interval. This shows the suitability and utility of these markers not only in genetic diversity studies but also in parentage testing and genome mapping projects.

Significant departure from HWE was shown at nine loci, possibly attributed due to some of the following reasons; presence of null alleles, heterozygosity deficiency, small sample size, population sub-structure (Wahlund effect) and inbreeding. Presence of null alleles is a common cause for HWE deviations (Pemberton et al., 1995). However, it is not possible to estimate the exact extent of null allele percentage, since there were no pedigree data available, and blood sampling was carried out with unrelated animals as well.

Inbreeding coefficient was calculated by two approaches; Weir and Cockerham method and Robertson and Hill method, and both showed mean values as 0.13. Moderate levels of inbreeding might be a factor that tends to deviate from the HWE. Peter et al. (2007) study showed inbreeding coefficient as 0.085 by Weir and Cockerham method. Bottleneck analysis revealed that Najdi did not undergo genetic bottleneck. Stepwise Mutation Model (SMM) is the most significant and conservative model deviations in favor of heterozygotic excess which truly represent the bottleneck. Najdi did not reveal heterozygotic excess in this study. In the present study, SMM revealed no heterozygote excess in Najdi population by Wilcoxon rank test also in both methods. The test results rely on the following assumptions; no population substructure prevailing in the population, no immigration and emigration, sample is representative of a defined population, and the loci are selectively neutral which is proved by the Ewens-Watterson test.

Conclusion

This is a holistic study on Najdi sheep found in the Kingdom of Saudi Arabia. The findings of this research

demonstrate fair degree of genetic diversity of Najdi sheep and it has comparable amount of genetic diversity with some of the studies carried in other parts of the world. Most of the markers used in this study are good for genetic diversity studies, quantitative trait loci (QTL) studies and linkage mapping studies. Some of the tested loci deviated from HWE, since they are not a natural population and not abiding by the Hardy-Weinberg conditions. There was no selection acting on any of the markers used in this study. Therefore, no 'genetic hitchhiking' was found in Najdi sheep. Bottleneck was not found in the recent past in Najdi sheep. Therefore, any unique alleles present in this breed may not have been lost. Inbreeding within the Najdi population was moderate, depicting the lack of proper management plans. So, it is necessary to consider an action plan to be drawn to conserve this sheep breed by the stake holders.

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