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Molecular genetic characterization of native sheep prevalent in Nagarjuna Sagar area of Telangana

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Abstract
The study was undertaken to understand the genetic variation of native sheep prevalent in Nagarjuna Sagar area of Telangana were genotyped using 10 microsatellite markers. A total number of 82 alleles were amplified in the present study across the 10 microsatellite loci. The number of alleles at each locus varied from a minimum of six (OarAE129, OarCP38) to a maximum of 11 (MAF 214) with a mean of 8.2 alleles. The mean number of effective alleles was
7.221. The mean observed and expected heterozygosity was 0.104 and 0.855, respectively. The overall mean inbreeding coefficient was 0.878. The FIS values ranged from 0.832 (OarCP34) to 0.941 (BM8125). The inbreeding estimates obtained in this study were all the positive indicating the high deficiency of heterozygotes. All the ten microsatellite loci (100%) were found to be highly polymorphic and the PIC values ranged from 0.762 (OarAE129) to 0.890 (MAF214). The mean PIC value for all the ten loci was found to be 0.836. The chi-square test revealed that all the ten loci were showing significant deviation from Hardy-Weinberg study contribute to the knowledge of genetic structure of this native sheep. The results revealed high genetic variation within the breed.

Keywords: Alleles, within population inbreeding estimate, hardy-weinberg equilibrium, heterozygosity, polymorphic information content

Introduction
India is a rich repository of sheep genetic resources with 44 recognized sheep breeds and ranks 3rd in the world with 74.26 million sheep population as per 20th Livestock Census. Sheep play an important role in the Indian agrarian economy with its multifaceted utility for meat, wool, skin and manure. The Southern peninsular region is endowed with mutton breeds like Nellore, Mandya, Deccani, Hassan, Bellary, Kenguri, Coimbatore, Madras Red etc. Sheep play an important role in economy of Telangana and ranks first with highest population (19.1 million) in the country (BAHS, 2019) [4]. The state is having highly productive sheep breeds like Deccani and Nellore.
In certain areas of Telangana *viz*., Nagarjuna Sagar region of Nalgonda, Nagarkurnool and in Suryapet districts, farmers rear a particular type of local sheep referred to as "Chukkajaalagorre". About 46,000 of these native sheep are present in Nalgonda district of Telangana which lies in Southern Telangana Agro-climatic region with semi-arid climate comprising of conditions like high temperature and humidity. The farmers in this area prefer these sheep due to their adaptability to adverse climatic conditions which are known for their heat tolerance, disease resistance and as they thrive well in harsh climatic and scarce feeding conditions.
The United Nations Food and Agricultural Organization has proposed a global Programme for the management of genetic resources based on molecular methodology for breed characterization (Bjornstad and Roed, 2001) [5] and this strategy places a strong emphasis on the use of molecular markers to aid in the conservation and assessment of endangered breeds, as well as to determine the genetic status of these breeds. Hence the present study is undertaken to characterize native sheep at molecular level with the aid of FAO-ISAG designated microsatellite markers and also to assess the genetic status of native sheep prevalent in the Nagarjuna Sagar area of Telangana.

Material and Methods
Of the 100 blood samples of native sheep, 30 were collected from Nagarkurnool (Amrabad, padra, Akkaram, Maddimadugu) district, 50 were collected from Nalgonda district, while the remaining 20 were collected from suryapet (Palakeedu, Mattampelle, Mellacheruvu) district.

DNA was extracted from blood samples following the phenol-chloroform method (Sambrook *et al.* 1989) [20]. The quantity of genomic DNA was measured by UV spectrophotometer and quality by electrophoresis on 0.8% agarose gels. A total of 30 microsatellite primer sets specific for sheep were used in the study as recommended by FAO (http://www.fao.org/dad-IS). PCR amplification was performed on Eppendorf thermal cycler. Each 12.5 µl PCR reaction mixture contained 1 µl of 100 ng of genomic DNA, 1.25 µl of 10xTaq buffer, 0.75 µl of 25 mM MgCl2, 0.25 µl of 10mM-dNTPs, 0.5 units of Taq polymerase with 0.6 µl of forward and reverse primers and final 0.755 µl of autoclaved milliQ water. The cycling protocol was -5min denaturing at 95 °C followed by 34 cycles of denaturation at 94 °C for 1min, annealing at different temperatures (depending on primers) for 30 sec, extension at 72 °C for 30 sec and the final extension at 72 °C for 5min. the amplified PCR products were electrophoresed on 2% agarose to resolve the bands. The bands of the gel were visualized under UV light of gel documentation system (Syngene).

Statistical Analysis

Microsatellite allele frequencies, effective number of alleles (Ne), polymorphism information content (PIC), test of Hardy-Weinberg equilibrium, observed (Ho) and expected heterozygosity (He) and F-statistics (FIS) were calculated using the POPGENE version 1.32 (Yeh *et al.*, 1999) [26]. Polymorphism information content was calculated using PIC calculator in the Google home page.

Results and Discussion

The values for mean number of alleles, effective number of alleles, observed and expected heterozygosity, Polymorphism information content, FIS and hardy-weinberg equilibrium are presented in Table 1.

In the present study, a total of 82 alleles were identified across all the ten microsatellite alleles under study. The number of alleles at a locus ranged from 6(OarAE129, OarCP38) to 11(MAF214) with a mean number of 8.2 alleles. The effective number of alleles ranged from 4.810 (OarAE129) to 9.970 (MAF214) with a mean value of 7.221. present study was higher than with the mean number of effective alleles reported by Radha *et al.* (2011) [19] in Kilakarsal (3.85) Sheep, Nanekarani *et al.* (2011) [15] in Karakul (6.75) sheep, Hepsibha *et al.* (2014) [9] in Coimbatore (3.37) sheep, Vani *et al.* (2017) [24] in Nellore (4.72) sheep, Amareswari *et al.* (2018) [2] in Deccani (5.529) and Nellore (5.620) sheep, Manjari *et al.* (2018) [13] in Nellore brown (6.78) sheep, whereas, Ghazy *et al.* (2013) [8] reported higher mean effective allelic number in Rahmani (11.38) and Ossimi (11.81) breeds of Egypt with a mean of 14.13.

Heterozygosity

The observed heterozygosity values in this native sheep ranged from 0.050 (BM8125) to 0.140 (OarCP34) with a mean value of 0.104, whereas, the expected heterozygosity ranged from 0.792 (OarAE129) to 0.900 (MAF214) with a mean value of 0.855. The observed heterozygosity values were less than the expected heterozygosity for all the loci in the present study which was in consonance with studies of Radha *et al.* (2011) [19], Musthafa *et al.* (2012) [14], Das and Demir (2015) [7], Vani *et al.* (2017) [24] in Nellore, Amareswari *et al.* (2018) [2] in Deccani and Nellore sheep, Manjari *et al.* (2018) [13] in Nellore brown sheep and Sharma *et al.* (2020) [21] in native sheep breeds. The observed genetic variation in this native sheep is much lower than the expected heterozygosity which indicates the significant deficiency of heterozygotes at almost all markers selected. The expected heterozygosity values noticed in the present study were almost in harmony with studies of Kumarasamy *et al.* (2009) [12] in Coimbatore sheep (0.8106), Arora *et al.* (2010) [3] in Ganjam sheep (0.623), Radha *et al.* (2011) [19] in Kilakarsal (0.618) sheep, Nanekarani *et al.* (2011) [15] in Karakul (0.831) sheep, Jyotsana *et al.* (2012) [10] in Patanwadi, Marwari and Dumba sheep breeds (0.70), Musthafa *et al.* (2012) [14] in Najdi sheep (0.67), Hepsibha *et al.* (2014) [9] in Coimbatore sheep (0.6255), Al-Atiyat *et al.* (2014) [1] in Jordanian sheep (0.678), Das *et al.* (2015) [7] in Turkey sheep breeds (0.734), Pons *et al.* (2015) [16] in Balearic sheep (0.69), Vani *et al.* (2017) [24] in Nellore (0.790) sheep, and Manjari *et al.* (2018) [13] in Nellore brown (0.840) sheep. Have recorded lower values for expected heterozygosity than the present study. Expected heterozygosity is considered to be a better estimate of the genetic variability while, the value obtained in the present study indicated that the population had retained several alleles and this reciprocates the existence of genetic variability in overall population of native sheep in the breeding tract.

Within population inbreeding estimate

The FIS values in the present study ranged from 0.832 (OarCP34) to 0.941 (BM8125). The mean value of heterozygote deficit (FIS) observed was 0.878 and the FIS values reported were positive across all the ten loci studied in this sheep. A positive value of inbreeding estimate values reported by Pramod *et al.* (2009) [17] in Vembur (0.295), Radha *et al.* (2011) [19] in Kilakarsal sheep (0.147), Musthafa *et al.* (2012) [14] in Najdi sheep (0.127), Vani *et al.* (2017) [24] in Nellore sheep (0.19), and Manjari *et al.* (2018) [13] in Nellore brown sheep (0.882), whereas, Qanbari *et al.* (2007) [18] in Afshari sheep (-0.020), Nanekarani *et al.* (2011) [15] in Karakul sheep (-0.197), Hepsibha *et al.* (2014) [9] in Coimbatore sheep (-0.0024) in two indigenous sheep breeds (-0.020) reported negative mean FIS value indicating the absence of inbreeding. Even though, only one blood sample from small flocks (flock size < 20) and a maximum of two samples from large flocks (flock size > 20) of native sheep and samples from only three to four flocks per village were collected, the higher values were recorded for FIS. Uncontrolled mating in sheep populations at the farmers level and the unequal sex ratio of breeding animals might have caused inbreeding. Further, breeding flocks mostly comprised of a dominant male and few numbers of females for almost more than three to four years and presumably most of the offspring born are inbred and the above practices that are being followed in the sheep flocks in sampled areas might be one of the causes of the possibility of heterozygote deficiency in this native sheep.

Polymorphism information content

The polymorphism information content (PIC) values for the ten loci ranged from 0.762 (OarAE129) to 0.890 (MAF214) in sheep. The mean PIC value (0.836) in the present study was almost in accordance with the findings of Kumarasamy *et al.* (2009) [12] in Coimbatore sheep (0.8106), Nanekarani *et al.* (2011) [15] in Karakul sheep (0.808), Surekha *et al.* (2018) [22] in Nellore Jodipi Sheep (0.819), Kavitha *et al.* (2015) [11] in Tiruchy Black sheep (0.844), Manjari *et al.* (2018) [13] in

Nellore brown sheep (0.824) in two indigenous sheep breeds (0.79), PIC values higher than in the present study were noticed by Ghazy *et al.* (2013) [8] in Egyptian sheep breeds (0.903), and mean PIC values lower than the present study were recorded in Patanwadi (0.66), Marwari (0.70) and Dumba (0.66, Jyotsana *et al.*, 2010) [10], Najdi (0.71; Musthafa *et al.*, 2012) [14], Sicilian sheep
(0.76; Tolone *et al.*, 2012), Coimbatore (0.5851; Hepsibha *et al.*, 2014) [9], Balochi (0.55), Rakhshani (0.57; Wajid *et al.*, 2014), Turkey Sheep (0.705; Das *et al.*, 2015) [7], Vani *et al.* (2017) [24] in Nellore sheep (0.753), Amareswari *et al.* (2018) [2] in Deccani (0.792) and Nellore (0.791) sheep breeds, The PIC values for all the ten microsatellite loci in the present study were more than 0.50 indicating the suitability of these markers for the genetic diversity analysis in this native sheep and also suggest the utility of the selected set of microsatellites in molecular characterization and there by the genetic variability of the investigated population can be exploited.

Hardy-weinberg equilibrium

The chi- square test values of all the 10 loci revealed that they were showing significant deviation from Hardy-Weinberg Equilibrium which was in similar with the findings of Nanekarani *et al.* (2011) [15] in Karakul sheep, Vani *et al.* (2017) [24] in Nellore sheep and Manjari *et al.* (2018) [13] in Nellore brown sheep. Population disequilibria similar to present study was also noticed in 5 loci out of 25 (Sharma *et al.*, 2010) [21] in Changthangi sheep, 17 out of 23 loci (Radha *et al.*, 2011) [19] in Kilakarsal sheep, 12 out of 28 loci (Calvo *et al.*, 2011) in Merino and Spanish Mouflon sheep, all 14 loci (Zahan *et al.*, 2011) [27] in Tsigai sheep poplation, 19 loci (Ghazy *et al.*, 2013) [8] in Egyptian sheep breeds, 12 out of 24 loci (Hepsibha *et al.*, 2014) [9] in Coimbatore sheep and only ten among the 25 microsatellites studied (Kavitha *et al.*, 2015) [11] in Tiruchy black sheep. This deviation of all the loci of this native Sheep from HWE in the present study may be attributed to the presence of low frequency null alleles segregating at all these loci. High positive FIS (within population inbreeding estimates) values and presence of population substructure (Wahlund effect) could be the other reasons for this deviation obtained at all loci.

Conclusion

With the result of the present study, it is concluded that the total of 82 alleles were detected in the native sheep with 8.200 as the mean observed number of alleles and 7.221 as the mean effective number of alleles. The mean observed heterozygosity (Ho), expected heterozygosity (He) values were 0.104 and 0.855, respectively with a mean PIC value of 0.836. High PIC values indicate more polymorphism and genetic diversity of the breed with high sustainability of the gene pool. All the microsatellite loci under study significantly deviated from the HWE with a high mean inbreeding estimate (FIS) of 0.878. Even though native sheep revealed high genetic diversity among sampled population with high He and PIC values (>0.5), the inbreeding (FIS) values estimated across all the loci studied indicated high inbreeding in native sheep population of Telangana. This might be attributed to genetic hitch-hiking effect, linkage with loci under selection and un observed null alleles. In addition to this, there are number of causes like subdivision of population leading to Wahlund effect, assortative mating, heterozygote deficiencies and consanguinity present in the population/sub-population. Although native sheep has no threat of extinction, the genetic diversity of unique sheep should be exploited by following breeding strategies like selective breeding, periodical exchange of rams, maintaining a nucleus breeding stock and supply of superior germplasm to the farmers.

Table 1: Observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He), within population inbreeding (FIS), polymorphic information content (PIC) and hardy-weinberg equilibrium (HWE) of native sheep of Telangana for 10 microsatellite markers

Locus	Na	Ne	Ho	He	FIS	PIC	HWE
BM8125	9	6.660	0.050	0.850	0.941	0.833	694.421***
HUJ616	8	7.716	0.090	0.870	0.897	0.856	558.386***
INRA063	10	9.179	0.090	0.891	0.899	0.880	751.333***
MAF214	11	9.970	0.090	0.900	0.900	0.890	806.055***
OarAE129	6	4.810	0.130	0.792	0.836	0.762	341.180***
OarCP34	7	6.083	0.140	0.836	0.832	0.814	416.937***
OarCP38	6	5.724	0.110	0.825	0.867	0.800	377.565***
OarFCB128	10	9.091	0.120	0.890	0.865	0.879	674.231***
OarHH47	7	6.297	0.120	0.841	0.857	0.820	451.169***
OarJMP29	8	6.680	0.100	0.850	0.882	0.833	546.275***
Mean	8.2	7.221	0.104	0.855	0.878	0.836	

***Significant ($p \leq 0.001$)

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