



Molecular genetic characterisation of Salem Black goats

K THILAGAM¹, J RAMAMOORTHY², S N SIVASELVAM³, S M K KARTHICKEYAN⁴ and P THANGARAJU⁵

Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 007 India

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Out of 124.36 million (Livestock Census 2003) goats available in India, majority proportion is constituted by non-descript goats. Kanniadu is a recognized breed of goat (Acharya 1982) but 3 distinct genetic groups, viz. Kodiadu, Pallaiadu and Salem Black are also present in Tamil Nadu (Mariadas 2003). Characterisation of these genetic groups is the first step in recognizing them as breeds. Jain *et al.* (2000) documented 'Kodiadu' as a new goat breed of Tamil Nadu by phenotypic characterisation. Mariadas (2003) reported the preliminary information on the presence, uniqueness and utility of Salem Black goats. Gopu (2002) reported systematic investigation on the distribution, phenotypic characteristics, biochemical and cytogenetic profile of Salem Black goats. However, genetic characterization is lacking. Hence, the present study was carried out with an aim of characterizing Salem Black goats at molecular level using microsatellite markers so as to elucidate the complete and comprehensive genetic structure for recognizing and declaring this genetic group as a new breed of goats in India.

Salem Black goats are distributed in Mettur and Omalur taluks of Salem; Pennagaram and Palacode taluks of Dharmapuri; and Bhavani taluk of Erode districts. The estimated population size was around 87, 000 in the breeding tract. The animals are tall, predominantly black and legs are lean and straight. The entire body is covered with black short and soft hairs except in neck and withers. Head is medium with broad forehead and black or gray tapering muzzle. Both sexes are horned with curvature upwards and backwards (Gopu 2002).

A total of 21 microsatellite markers were used for

molecular characterisation of Salem Black goats in the present study (Table 1) as per the integrated programme for the global management of genetic resources (Project MoDAD, <http://www.fao.org/dad-is>) using microsatellite methodology for breed characterization (FAO 2004). The investigation was carried out on 50 unrelated animals of Salem Black goats. Genomic DNA was isolated from 5 ml of blood by a rapid non-enzymatic method (Lahiri and Nurnberger 1991). The amplification reactions were carried out using a programmable thermal cycler in a reaction volume of 20µl. Each 20µl PCR reaction contained 50 to 100ng of template DNA; 5pmol of each primer; 500µM each of dNTPs; 0.75 unit of TaqDNA polymerase; and 1µl of 1.5 mM MgCl₂. The primer annealing for 45 sec at the desired temperatures of 50, 53, 55 or 58°C was adopted. The amplified PCR products were electrophoresed in 6% denaturing urea polyacrylamide gel at a constant voltage of 1300 for 1 to 2 h depending upon the size of PCR products and then silver-stained (Comincini *et al.* 1995).

The gels were blotted and dried at 80°C for 1h. The alleles were scored manually and analysed by software aided gel documentation system. The exact allele sizes were determined by direct comparison with adjacent PCR bands and 10bp ladder marker. Allele frequencies were estimated by direct counting from the genotypes of the goats. The polymorphism information content (PIC) was calculated using the individual frequencies in which the alleles occur at each locus (Nei 1978). The observed and expected heterozygosities were estimated (Nei 1973) for the 21 microsatellite loci. The expected number of genotypes was compared with the observed genotypes in a χ^2 test for goodness of fit to assess whether the study population was in Hardy-Weinberg equilibrium (HWE).

The results of the microsatellite analyses of Salem Black goats in terms of number of alleles observed, range of alleles size with frequencies, polymorphism information content and heterozygosity are tabulated (Table 1). In total, 136 alleles were observed for the 21 microsatellite loci with a mean of 6.5 per locus. The number of alleles per locus ranged from 4 (ILSTS002, ILSTS004, ILSTS040, ILSTS049 and

Present address: ¹Veterinary Assistant Surgeon (jramamoorthi@yahoo.com), Namakkal 637 001.

²Research Officer (kthilagam@gmail.com), Royal Darwin Hospital, Casuarina, NT 0811, Australia.

³Professor (snsivaselvam@hotmail.com), ⁴Associate Professor (kannikarthi@yahoo.co.in), Department of Animal Genetics and Breeding, Madras Veterinary College, Chennai 600 007.

⁵Vice-Chancellor (Retd.) (ptrajuagb@yahoo.com), Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu 600 051.

Table 1. Microsatellite alleles, polymorphism information content and heterozygosity values in Salem Black goats

Microsatellite marker	No. of alleles	Allele size range (bp)	Allele frequency range	PIC	Heterozygosity	
					Observed	Expected
ILSTS001	7	98 – 126	0.0208 – 0.3854	0.6640	0.9583	0.7135
ILSTS002	4	126 – 144	0.0666 – 0.4440	0.6021	0.9771	0.6641
ILSTS004	4	94 – 108	0.2209 – 0.2791	0.6993	1.0000	0.7466
ILSTS008	7	120 – 160	0.0106 – 0.3085	0.7178	0.9149	0.7577
ILSTS019	7	138 – 162	0.0185 – 0.4259	0.6337	0.8880	0.6869
ILSTS022	8	176 – 202	0.0208 – 0.2500	0.8085	0.9583	0.8302
ILSTS028	7	120 – 140	0.0435 – 0.2391	0.7965	1.0000	0.8209
ILSTS029	9	140 – 184	0.0341 – 0.2386	0.8424	0.8863	0.8541
ILSTS038	7	156 – 176	0.0222 – 0.3111	0.7448	0.9777	0.7771
ILSTS040	4	164 – 172	0.0532 – 0.4468	0.6123	0.9570	0.6720
ILSTS044	9	132 – 180	0.0111 – 0.2889	0.7833	0.9777	0.8079
ILSTS049	4	178 – 184	0.0106 – 0.4574	0.5710	0.9361	0.6470
ILSTS052	8	128 – 144	0.0300 – 0.3222	0.7462	0.9166	0.7732
ILSTS058	4	110-120	0.0813 – 0.4186	0.6352	0.9302	0.6911
ILSTS059	8	192 – 206	0.0340 – 0.2045	0.8233	0.9090	0.8423
ILSTS060	5	96 – 104	0.0119 – 0.4642	0.5885	1.0000	0.6516
ILSTS065	6	138 – 170	0.0109 – 0.3696	0.6838	0.8478	0.7302
ILSTS072	7	94 – 108	0.0244 – 0.2683	0.7761	0.7804	0.8043
ILSTS078	8	74 – 122	0.0109 – 0.2609	0.8034	0.8913	0.8255
ILSTS082	7	112 – 126	0.0745 – 0.2469	0.8166	1.0000	0.8380
ILSTS087	6	112-150	0.0256 – 0.2820	0.7230	0.9523	0.7632

ILSTS058) to 9 (ILSTS029 and ILSTS044). The allele sizes ranged from 74bp (ILSTS078) to 206bp (ILSTS059). The mean number of alleles observed in the present study was higher than that reported in Sirohi (4.12), Jamnapari (4.00) and Barbari (3.37) goat breeds using cattle-specific microsatellite markers (Ganai and Yadav 2001), but was similar to the mean number of alleles observed in Barbari goats (6.3; Ramamoorthi *et al.* 2009) with goat-specific microsatellite markers. On the contrary, it was lower than the mean number of alleles observed for Kanniadu goat, the only recognized breed of Tamil Nadu (7.9, Thilagam *et al.* 2006) and for Tellicherry goat breed (7.1; Ramamoorthi *et al.* 2006) with goat-specific microsatellite markers. The allele sizes obtained in the present study are also lower than those observed in Kanniadu (90 to 222 bp, Thilagam *et al.* 2006); Barbari (88 to 220 bp) and Tellicherry goats (92 to 298 bp) (Ramamoorthi *et al.* 2006). The frequency of the alleles ranged from 0.0106 (ILSTS008) to 0.4642 (ILSTS060) in Salem Black goats.

The polymorphism information content (PIC) values were generally high, ranging from 0.5710 (ILSTS049) to 0.8424 (ILSTS029) with a mean of 0.7177. Except ILSTS059, ILSTS060, ILSTS065 and ILSTS072 loci, the differences between observed and expected number of genotypes were significant ($P \leq 0.01$) for the remaining 17 loci indicating population disequilibria. This is almost similar to that of Kanniadu (0.7499; Thilagam *et al.* 2006) and Tellicherry goats (0.7309, Ramamoorthi *et al.* 2006). On the contrary, the mean PIC value was higher than those of Barbari (0.6862,

Ramamoorthi *et al.* 2009) and of Sirohi, Jamnapari and Barbari breeds (overall mean PIC value of 0.48 Ganai and Yadav 2001). When compared with other Indian goat breeds, the PIC estimated in the present study concurred with Bengal and Chegu breeds (0.79 and 0.78, respectively; Behl *et al.* 2003). Based on the PIC values, it was proved that the microsatellite markers were highly polymorphic and could be used for molecular characterization of goat breeds.

The present population of Salem Black goats was in HWE only at 4 loci. The deviation of about 81% of the loci (17 out of 21) might be due to many causes such as selection, genetic drift and small sample size. Apart from these, existence of “null” alleles, high mutation rate and size homoplasy of microsatellite loci might have contributed to the disequilibria. Unlike diallelic system, the attainment of equilibrium would take longer time as the nature of inheritance of microsatellites follow multiple allelic fashion. Similar disequilibria were also reported at majority of loci i.e., 17 loci in Kanniadu (Thilagam *et al.* 2006), 19 loci in Barbari and 15 loci in Tellicherry (Ramamoorthi *et al.* 2006).

The heterozygosity is an appropriate measure of genetic variability within a population. The observed heterozygosity ranged from 0.7804 to 1.0000 while the expected heterozygosity ranged from 0.6470 to 0.8541 across the microsatellite markers studied. The range of observed heterozygosity obtained in the study is comparable with that of Kanniadu (0.7142 to 1.0000, Thilagam *et al.* 2006), while it was lower than those of Barbari and Tellicherry (0.8478 to 1.0000 and 0.8444 to 1.0000, respectively; Ramamoorthi *et*

al. 2006, 2009) goats. The high expected heterozygosity estimated in the present study (0.6470 to 0.8541) is similar to that of other goat breeds of India viz. Bengal and Chegu goats (0.69 and 0.66, respectively, Behl *et al.* 2003), Kanniadu (0.6390 to 0.8702, Thilagam *et al.* 2006), and Barbari and Tellicherry (0.6208 to 0.8509 and 0.6445 to 0.8504 respectively; Ramamoorthi *et al.* 2006, 2009). The higher heterozygosity observed in the present investigation had resulted from retention of many alleles in small frequencies. Because of higher heterozygosity, there is further scope for improvement of Salem Black goats.

To conclude, the present study showed that all the microsatellite markers used were highly polymorphic and informative for molecular characterization. The population has high amount of variations with respect to the loci screened. Based on the results, it is clearly understood that Salem Black goats also showed similar pattern of distribution of alleles and polymorphisms as reported in Kanniadu breed of Tamil Nadu; Tellicherry of Kerala and Barbari of Uttar Pradesh and Rajasthan. Our results showed that genetic structure of the Salem Black goats is sufficient enough to qualify for recognition as a new goat breed of India.

SUMMARY

The present investigation was carried out to study the genetic architecture of Salem Black goats, a distinct genetic group of goats in Tamil Nadu, which needs to be recognized as a breed. The genomic DNA samples from 50 unrelated Salem Black goats were evaluated with a panel of 21 microsatellite markers for genetic characterization. In these animals, the number of alleles ranged from 4 to 9 with a mean of 6.5 / locus. The sizes of alleles and their frequencies ranged from 74 to 206bp and from 0.0106 to 0.4642 respectively. Polymorphism information content ranged from 0.5710 to 0.8424 with a mean PIC of 0.7177. Except for four loci, the population was not in Hardy-Weinberg equilibrium. The observed heterozygosity ranged from 0.7804 to 1.0000 while the expected heterozygosity ranged from 0.6470 to 0.8541. The study revealed that there is high amount of genetic variation present in the population with the defined levels of heterozygosity and polymorphism information content. Hence, this distinct population of Salem Black goats could be recognized as a breed of goats in Tamil Nadu.

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REFERENCES

- Acharya R M. 1982. *Sheep and Goat Breeds of India*. FAO Animal Production and Health Paper 30, pp. viii+190, FAO, United Nations, Rome.
- Behl R, Sheoran N, Behl J, Vijh R K, Tania M S. 2003. Analysis of 22 heterologous microsatellite markers for genetic variability in Indian goats. *Animal Biotechnology* **14**: 167–75.
- Comincini S, Leone P, Redaelli I, De Giuli L, Zhang Y and Ferretti L. 1995. Characterisation of bovine microsatellites by silver staining. *Journal of Animal Breeding and Genetics* **112**: 415–40.
- Ganai N A and Yadav B R. 2001. Genetic variation within and among three Indian breeds of goat using heterologous microsatellite markers. *Animal Biotechnology* **12**: 121–36.
- Gopu P. 2002. 'Genetic characterisation of Salem Black goat.' M.V.Sc. thesis, Tamilnadu Veterinary and Animal Sciences University, Chennai, India.
- Jain A, Sahana G, Kandasamy N and Nivsarkar A E. 2000. Kodi Adu – A new goat breed of Tamil Nadu. *Indian Journal of Animal Sciences* **70**: 649–51.
- Lahiri D K and Nurnberger Jr J I. 1991. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research* **19**: 5444.
- Livestock Census. 2003. Government of India.
- Mariadas B. 2003. *Goat Breeds of Tamil Nadu and their Management Practices. A Monograph*. pp.1–11. Gunaseeli Publishers, Trichy (in Tamil).
- Nei M. 1973. The theory and estimation of genetic distance. *Genetic Structure of Populations*. pp.45–54. (Ed.) Morton N E. University of Hawaii Press, Honolulu.
- Nei M. 1978. Estimation of average heterozygosity and genetic distance from small number of individuals. *Genetics* **89**: 583–90.
- Ramamoorthi J, Sivaselvam S N, Subramanian A, Kumarasamy P, Karthickeyan S M K and Thangaraju P. 2006. Genetic structure of Tellicherry goats (*Capra hircus*) based on microsatellite markers. *Online Journal of Veterinary Research* **10**: 156–61.
- Ramamoorthi J, Thilagam K, Sivaselvam S N and Karthickeyan S M K. 2009. Genetic characterisation of Barbari goats using microsatellite markers. *Journal of Veterinary Science* **10**: 73–76.
- Thilagam K, Ramamoorthi J, Sivaselvam S N, Karthickeyan S M K and Thangaraju P. 2006. Kanniadu goats of Tamilnadu, India: genetic characterization through microsatellite markers. *Livestock Research for Rural Development* **18** Article no. 149. (http://www.cipav.org.co/lrrd/lrrd18/10/thil_18149.htm)