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Ramnad White sheep—Phenotypic and genetic characterization

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The Southern Peninsular region of India is home to 14 descript sheep breeds out of which 7 (Madras Red, Mecheri, Ramnad White, Vembur, Nilgiri, Coimbatore and Tiruchy Black) are found in Tamil Nadu (Ganeskale and Rathnasabapathy 1973, Acharya 1982). Ramnad White is an important mutton sheep breed of southern Tamil Nadu, mainly distributed in Ramanathapuram, Sivagangai and adjoining areas of Thoothukudi district. Very scanty information is available on the morphological and genetic evaluation/characterization of this breed (Ganeskale and Rathnasabapathy 1973, Acharya 1982, Ravimurugan and Devendran 2009). In the present study the phenotypic as well as the genetic characterization of the Ramnad White breed was carried out. The genetic characterization based on microsatellite markers complements the phenotypic/ morphological evaluation in order to assess the status of the Ramnad White sheep prevailing in its distribution area. The results from this study will enable the outlining of conservation and improvement programmes/policies for this important sheep breed.

Phenotypic characterization: The distribution of the breed was identified through discussions with the various officers of the Animal Husbandry Department. Survey was conducted in Ramanathapuram, Thirupullani, Mudugalathur, Rameshwaram, Boogalor, Paramagudi, Kamuthi and Raja-singamangalam blocks of Ramnad/Ramanathapuram and Iiayangudi, Manamadurai and Tiruppuvanam blocks of Sivagangai districts for phenotypic characterization of this breed under field conditions. Information on morphological characters and performance parameters of 473 Ramnad White sheep was collected through interaction with the farmers, personal observations and on the spot recording from 28 farmers' flocks having more than 3000 animals. The body measurements were taken with a measuring tape after making the animal stand squarely on an even ground. The body weights were recorded by weighing the animals with a weighing machine and age of the animals was recorded by observing number of teeth.

Genotyping with microsatellite markers: Genomic DNA was isolated from blood samples of unrelated animals using the standard phenol chloroform extraction method (Sambrook *et al.* 1989). Microsatellite markers (24) recommended for ovines were selected for the analysis of genetic diversity in 57 genomic DNA samples of Ramnad White sheep. The forward primer for each marker was fluorescently labelled with either FAM, NED, VIC or PET dye. Microsatellites were amplified in a reaction mix containing approximately 100ng of genomic DNA, 100µM of each dNTP, 5pM of each primer, 1 unit of *Taq* DNA polymerase, 1.5 mM $MgCl_2$ and 1x buffer. A common "Touchdown" PCR programme was used for amplification of all the 24 markers (Bradley *et al.* 1997). Amplification was confirmed on 2% agarose gel and the genotyping was carried out on ABI 3100 automated DNA sequencer using LIZ 500 as internal size standard. Allele sizing was done using GENEMAPPER software.

Statistical analysis: The GENALEX-6 (Peakall and Smouse 2006) software was used to determine allele frequencies, observed and effective number of alleles, observed heterozyosity, expected heterozygosity and within breed heterozygote deficiency (F_{IS}). The tests for deviations from the Hardy-Weinberg equilibrium (HWE) were derived using GENEPOP-3.2a (Raymond and Rousset).

Habitat: Ramnad White sheep are distributed in Ramnathapuram, Sivagangai district and neighbouring areas of Tirunelveli district of Tamil Nadu. Ramanathapuram is located between 9° 05' and 9° 50' North Latitude and between 78° 10' and 79° 27' East Longitude. It covers the geographical area of 4175.00 sq.km. Paddy, sorghum, *cumbu* (*Pennisetum glaucum*), millets, pulses, cotton, groundnut, sunflower, gingelly, chillies, coriander, coconut and sugarcane are grown in this district. The flock purity of the breed is above 95% in most of the flocks surveyed.

Morphological characterization: Physical characteristics were established on 40 rams, 250 ewes, 75 male lambs and

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Figs 1–3. **1.** Ramnad White ewe. **2.** Ramnad White ram. **3.** Ramnad White flock.

108 female lambs. The animals are predominantly white with black belly and extremities or black spots around lips, eyes, ears, lower jaw and legs (Figs 1–3). Black marking is visible from the inner canthus of the eye to the base of the horn. The legs below the hock/knee joints are black. In the flocks about 1% of animals are completely white. The animals are hardy, strong with well proportioned frame, a broad and deep chest and straight topline. The ears are medium-sized and directed outward and downward, forehead is narrow and straight noseline. Males are horned (98%) and females are polled (only 10% of ewes had horn). Horns are thick, corrugated and curved in rams. Horn length was 30.7±1.81 and 10.9±1.42 cm in rams and ewes. The females have medium sized udder, well demarcated from the body with well defined teats. The tail is thin and short, coarse fleece covers the body. Body measurements were recorded on 290 animals of both the sexes. The averages of various body measurements are presented in Table 1. Average body weight was 43.9±1.07 and 31.5±0.26 kg in rams and ewes respectively. The rams exhibited higher estimates for all the parameters except ear length, which was slightly higher in ewes. Ganesakale and Rathnasabapathy (1973) reported lower body weight for Ramnad White rams and ewes.

Management: A typical flock consists of about 113 animals with 3 rams, 1 adult male, about 82 ewes and 27 lambs and hoggets. Acharya (1982) reported the average flock size of 52 animals (2 rams, 34 ewes and 16 lambs) with a range of 10 to 185 animals. The sheep farmers mainly belonged to Yadav and Muslim communities. Other livestock maintained by the farmers are cattle, goat, fowl, duck and turkey. The ram to ewe ratio is 1: 20 to 30. Sheep are housed in separate, fenced enclosures or in the open. The flocks are taken out for grazing at 9–10 AM and return at 6:30–7 PM. A distance of about 10 km is covered in a day during grazing; the shepherds were males of 30–70 years of age. The sheep are maintained only on grazing but young lambs are provided with neem leaves (*Azadirachta indica*). The lambs are sheltered in small housing structures named *kudupoo* or *kodapoo* in local language to protect them from predators and high temperature during summer. This structure is made of wooden sticks covered with palmyra tree leaves. In one *kudupoo/kodapoo,* 5–10 young lambs can be housed. The neem leaves are hung inside the *kudupoo* for feeding the lambs (Fig. 4). Shearing is done before rainy season to maintain cleanliness and comfort of the animal.

Health management: Vaccination is done against sheep pox, anthrax, enterotoxaemia, foot-and-mouth disease and *Peste des Petits Ruminants* but not against blue tongue which is also a prevalent disease. The morbidity results into substantial economic losses. The sheep farmers use anthelmintics (albendazole, fenbendazole, oxyclosonide ivermectin etc.) once a quarter. Mortality ranged from 1.2 to 21% in adults and 2.6 to 50% in lambs. Still birth/abortion was reported in 1–2 cases.

Reproduction and breeding: Rams are selected from the flock itself based on conformation, breed characteristics, height and good health. Age at first breeding was 12–24 months with a breeding life of 4–5 years. In case of ewes, age at first lambing was 18–24 months with a lambing interval of 12–15 months and 5–6 lifetime lamb production. September-October was main lambing season with a lambing per cent of 75 to 100.

Fig. 4. Housing (kudupoo/kodapoo) for Ramnad White lambs

Table1. Body biometry in Ramnad White sheep (no. of samples in brackets)

Character	Rams		Ewes	
	Mean±SE	Range	Mean±SE	Range
Body weight (kg)	43.9±1.07 (40)	29–56.0	31.5±0.26 (248)	21–43.5
Length (cm)	72.3±0.99 (40)	61–94	65.5±0.30 (250)	53–87
Height (cm)	81.8±0.57 (40)	72–89	74.4±0.20 (250)	63–85
Chest-girth (cm)	84.0±0.93 (40)	66–92	75.8±0.31 (250)	52–90
Paunch-girth (cm)	79.8±1.10 (40)	65–96	74.0±0.38 (250)	60–93
Ear-length (cm)	14.7±0.23 (40)	11–18	14.8±0.13 (249)	7–25
Face-length (cm)	20.7±0.33 (40)	13–24	19.0±0.12 (250)	13–24
Horn-length (cm)	30.7±1.81 (39)	3–49	10.9±1.42 (31)	3–42
Tail-length (cm)	10.7±0.28 (39)	6–15	10.1±0.10 (250)	6–16

Utility: Ramnad White sheep are primarily maintained for mutton. Male lambs are sold at 5–6 months of age @ ₹ 1500–2000. Healthy female lambs are reared for breeding, old ewes are disposed for slaughter. Rams are sold for slaughter at a price of ₹ 4000 to 5000. Selling price of the animals is based on body size, body weight and arbitrary body score like back fat thickness determined by butchers/ middlemen. Milk production is about 250–400 ml during peak lactation period with a lactation length of about 2 to 5 months. Lambs are allowed to suckle milk until weaning. General milking is not practiced by all the farmers. Sheep droppings are utilized by penning the sheep in the owner's field. The landless sheep farmers charge from the owners of the agricultural fields in lieu of fertilization of the land by penning the sheep flocks (₹ 0.75 to ₹ 1.00 / night/sheep).

Genetic characterization: Genotypic data from 24 microsatellites was used to assess the genetic structure of Ramnad White sheep. Parameters of genetic variability are included in Table 2. A total of 232 alleles were detected across the 24 analyzed microsatellite loci. All the loci were observed to be polymorphic and also exhibited high level of genetic variability as revealed by wide range of alleles, which varied from 3 (BM6506) to 16 (INRA63, OarHH47) with an average 9.667. The effective number of alleles ranged between 2.065 (BM6506) and 8.345 (HSC). The estimates of allele diversity were higher than those reported for Indian sheep of southern region characterized earlier (Sharma *et al.* 2006, Girish *et al.* 2007, Pramod *et al.* 2009, Radha *et al.* 2011). In these reports the analysis was done on 6% denaturing PAGE followed by silver staining, hence direct comparisons cannot be made. However, the observed values were comparable with those reported for other Indian and exotic sheep breeds analyzed by the same method (Peter *et al.* 2007, Quiroz *et al.* 2008, Alvarez *et al.* 2009, Jyotsana *et al.* 2010, Arora *et al.* 2010, Arora *et al.* 2011a, b, c).

Heterozygote deficiency analysis revealed significant deviation from HWE ($P < 0.001$) at 8 loci (Table 2). The exact basis of this departure is difficult to explain however the presence of low frequency null alleles segregating at these loci may be a possible reason. Presence of population substructure (Wahlund effect) may also lead to deviation from HWE. Absence of significant deviations from HWE across all the loci within the population suggested no evidence of linkage between loci. Subsequent analyses were, therefore, carried out on the basis that HWE prevailed in the investigated population (Marshall *et al.* 1999).

Observed heterozygosity ranged from 0.167 (CSSM47) to 0.982 (MAF214) and expected heterozygosity from 0.516 (BM6506) to 0.880 (HSC) across the 24 microsatellite loci. The results showed that Ramnad White sheep possesses a high level gene diversity (mean expected heterozygosity), indicating that the population has retained the presence of several alleles although at a small frequency. This implies that the genetic variability in Ramnad White sheep might be used in planning breeding strategies for this breed. The average observed (0.708) and expected heterozygosity (0.759) of Ramnad White sheep were relatively similar to other domestic sheep breeds investigated earlier (Tapio *et al.* 2005, Baumung *et al.* 2006, Peter *et al.* 2007, Legaz *et al.* 2008, Jyotsana *et al.* 2010, Arora *et al.* 2011a, b).

The within population inbreeding estimate (F_{IS}) revealed a 6.8% deficit of heterozygotes in Ramnad White sheep. Significant heterozygotes deficiencies were reported in several earlier investigated Indian sheep breeds (Radha *et al.* 2011, Arora *et al.* 2011b). Although the main cause for high genetic homogeneity or lack of heterozygotes in Ramnad White sheep breed might be due to inbreeding (overall positive F_{IS} value), the possibility of Wahlund effect (population substructure) cannot be ruled out due to collection of samples from different breeding flocks i.e. different villages in the same area. Further, the relatedness of few samples otherwise deemed unrelated during collection may not be denied due to non availability of pedigreed data under field conditions.

Our results contribute to the knowledge of phenotypic and genetic structure of Ramnad White sheep and revealed that substantial genetic variability, as evident from the high allele and gene diversity values, is present in Ramnad White sheep breed. Although the Ramnad White sheep population revealed low level of inbreeding, implementation of suitable

Table 2. Genetic variability indices across 24 microsatellite markers in Ramnad White sheep

Locus	Number of alleles		Heterozygosity		F_{IS}	P value
	Observed (Na)	Expected (Ne)	Observed (Ho)	Expected (He)		
BM0757	5	3.756	0.772	0.734	–0.052	0.806
BM0827	5	3.549	0.649	0.718	0.096	0.193
BM6506	3	2.065	0.368	0.516	0.286	0.000
BM6526	11	3.936	0.737	0.746	0.012	0.000
BM8125	7	2.450	0.667	0.592	–0.126	0.278
CSRD247	11	5.544	0.772	0.820	0.058	0.635
CSSM31	13	7.654	0.579	0.869	0.334	0.000
CSSM47	9	3.000	0.167	0.667	0.750	0.000
HSC	13	8.345	0.800	0.880	0.091	0.371
INRA63	16	4.813	0.807	0.792	–0.019	0.948
MAF214	6	2.936	0.982	0.659	–0.490	0.000
OarAE129	13	3.818	0.754	0.738	–0.022	0.675
OarCP20	6	2.411	0.526	0.585	0.101	0.402
OarCP34	7	3.965	0.825	0.748	–0.103	0.108
OarCP49	10	7.117	0.877	0.859	–0.021	0.516
OarFCB048	10	6.637	0.825	0.849	0.029	0.673
OarFCB128	9	3.838	0.807	0.739	–0.091	0.935
OarHH35	15	7.668	0.855	0.870	0.017	0.000
OarHH41	6	3.606	0.649	0.723	0.102	0.326
OarHH47	16	6.492	0.789	0.846	0.067	0.000
OarHH64	9	5.136	0.510	0.805	0.366	0.000
OarJMP08	10	5.515	0.782	0.819	0.045	0.054
OarJMP29	14	7.478	0.873	0.866	–0.007	0.015

breeding strategies is suggested to reduce and prevent further inbreeding. For effective management and improvement of this breed, market oriented production policies conjointly with the farmers need to be developed.

SUMMARY

The phenotypic and genetic characterization of Ramnad White, an important mutton type sheep breed of Tamil Nadu state is presented. The animals are medium sized with hairy fleece and predominantly white coat colour. Average flock size is 113. Measurements were recorded for body weight, body length, height at withers, chest girth, paunch girth, ear length, horn length and tail length in 290 adult animals. The genetic variability in 57 DNA samples of Ramnad White breed using microsatellite markers was assessed in terms of observed number of alleles, observed heterozyosity, expected heterozygosity and within breed heterozygote deficiency (F_{IS}). A total of 232 alleles were identified across the 24 markers. The average estimates of allele diversity (9.667), observed heterozygosity (0.708) and gene diversity (0.759) elucidated substantial genetic diversity within Ramnad White breed. Within population inbreeding estimates (F_{IS}) for investigated population (0.068) showed a low rate of inbreeding.

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REFERENCES

Acharya R M. 1982. *Sheep and Goat Breeds of India*. FAO Animal Production and Health Paper 30. FAO, United Nations, Rome, Italy. 1–190.

Alvarez I, Traore A, Tamboura H H, Kabore A, Royo L J, Fernandez I, Ouedraogo-Sanou G, Sawadogo L and Goyache F. 2009. Microsatellite analysis characterizes Burkina Faso as a genetic contact zone between Sahelian and Djallonke sheep. *Animal Biotechnology* **20:** 47–57.

Arora R, Bhatia S and Mishra B.P. 2010. Genetic structure analysis of Deccani sheep-A major breed from Maharashtra. *Indian Veterinary Journal* **87** (11): 1109–11.

Arora R J, Bhatia S, Mishra B P, Jain A and Prakash B. 2011a. Diversity analysis of sheep breeds from Southern peninsular and Eastern regions of India. *Tropical Animal Health and Production* **43** (2): 401–08.

Arora R, Bhatia S, Yadav D K and Mishra B P. 2011b. Current genetic profile of sheep breeds/populations from Northwestern semi arid zone of India. *Livestock Science* **135**: 193–98.

Arora R, Bhatia S, Mishra B P and Joshi B K. 2011c. Population structure in Indian sheep ascertained using microsatellite information. *Animal Genetics* **42** (3): 242–50.

Baumung R, Cubric-Curik V, Schwend K, Achmann R and Sölkner

J. 2006. Genetic characterization and breed assignment in Austrian sheep breeds using microsatellite marker information. *Journal of Animal Breeding and Genetics* **123**: 65–271.

Bradley D G, Fries R, Bumstead N, Nicholas F W, Cothran E G, Ollivier,L and Crawford A M 1997. *Report of an Advisory group of the International Society of Animal Genetics.* Contribution to assist FAO prepare for the Modad Project, DADIS. FAO. 1–15.

Ganeskale D and Rathnasabpathy V. 1973. Sheep breeds of Tamil Nadu. *Cherion* **2**:146–55.

Girish H, Sivaselvam S N, Karthickeyan S M K and Saravanan R. 2007. Molecular characterization of Nilagiri sheep (*Ovis aries*) of south India based on microsatellites. *Asian- Australasian Journal of Animal Science* **20** (5): 633–37.

Jyotsana B, Jakhesara S, Prakash V, Rank D N and Vataliya P H. 2010. Genetic features of Patanwadi, Marwari and Dumba sheep breeds (India) inferred by microsatellite markers. *Small Ruminant Research* **93**: 57–60.

Legaz E, Alvarez I, Royo L J, Fernandez I, Gutierrez J P and Goyache F. 2008. Genetic relationships between Spanish Assaf (Assaf.E) and Spanish native dairy sheep breeds. *Small Ruminant Research* **80**: 39–44.

Marshall T C, Sunnucks P, Spalton J A, Greth A and Pemberton J M. 1999. Use of genetic data for conservation management: the case of the Arabian oryx. *Animal Conservation* **2**: 269–78.

Peakall R and Smouse P E. 2005. GENALEX-6: Genetic Analysis in Exel. Population genetic software for teaching and research. *Molecular Ecology Note* **6**: 288–95.

Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G and Erhardt G. ECOGENE consortium 2007. Genetic diversity and subdivision of 57 European and Middle-Eastern sheep breeds. *Animal Genetics* **38**: 37–44.

Pramod S, Kumarasamy P, Rosalyn Mary Chandra A, Sridevi P and Rahumathulla P S. 2009. Molecular characterization of Vembur sheep *(Ovis aries)* of south India based on microsatellites. *Indian Journal of Science and Technology* **2** (11): 55–58.

Quiroz J, Martinez A M, Zaragoza L, Perezgrovas R, Vega-Pla J L and Delgado J V. 2008. Genetic characterization of the autochthonous sheep populations from Chiapas, Mexico. *Livestock Science* **116**: 156–61.

Radha P, Sivaselvam S N, Kumarasamy P and Kumanan K. 2011. Genetic diversity and bottleneck analysis of Kilakarsal sheep by microsatellite markers. *Indian Journal of Biotechnology* **10**: 52–55.

Ravimurugan T and Devendran P. 2009. Body measurements and body weight of Ramand White sheep. *Indian Journal of Small Ruminants* **15** (2): 266–67.

Raymond M and Rousset F. 1995. GENEPOP-3.2a, Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248–49.

Sambrook J, Fritsch E F and Maniatis T. 1989. *Molecular Cloning: A Laboratory Manual.* 2nd edn. Cold Spring Harbor Press, New York.

Sharma R, Pandey A K, Kumar D, Jain A, Malik G, Gour D S and Ahlawat S P S. 2006. Genetic variation analysis of Hassan sheep population using microsatellite markers. *Korean Journal of Genetics* **28**: 43–51.

Tapio I, Tapio M, Grislis Z, Holm L E, Jeppsson S, Kantanen J, Miceikiene I, Olsaker I, Viinalass H and Eythorsdotti E. 2005. Unfolding of population structure in Baltic sheep breeds using microsatellite analysis. *Heredity* **94**: 448–56.