Sheep Genetic Resources of India

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Monograph 33, 2006

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Published by : **Dr. S.P.S. Ahlawat**
Director
National Bureau of Animal Genetic Resources
P.O. Box. - 129, Karnal - 132001 (Haryana) India

Cover Design : **Dr. Anand Jain & Dr. Vikram Singh**

Printing : **Intech Graphics**
51-A, Model Town, Karnal - 132 001
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Introduction

Present day sheep breeds or populations in India are as a result of long term natural selection for adaptation to the agro-ecology of their habitat accompanied by man made selection to meet social and economic requirements. Since agro-climatic conditions of India are varied, therefore the breeds developed under these conditions also vary not only in their morphology and genetic makeup but also in their adaptation, feed and fodder utilization, reproduction, growth, and production characteristics. The study of animal genetic resources in their natural habitat is important because not only their contribution to the national gross domestic production is substantial and important but also they are an integral and indispensable component of agriculture in India. Karnataka is one of the states in India where sheep is an important livestock species and contributes substantially to the income of the farmers, particularly the landless labourers and the marginal farmers. Of the four recognized sheep breeds, Kenguri is well adapted to the red soils and agroclimatic conditions of northern Karnataka. The present document describes Kenguri sheep of Karnataka in its native tract. The information are based on the work undertaken at NBAGR but relevant information from the literature has also been incorporated to make the document comprehensive.

Methodology

A survey was undertaken in Kustagi, Gangavati, Yelburga and Koppal Taluka of Koppal district, Lingasugur, Deodurga and Sindhnur Taluka of Raichur district and Hungund Taluka of Balakot district covering a total of 86 farmers' flocks to collect information related to morphological characterization and evaluation of Kenguri sheep. Information on feeding, breeding, management practices and utility pattern of the sheep

and socio-economic conditions of the farmers in the area were collected by personal observations and interaction with farmers. Information on reproductive performance and production traits were collected through interaction with the farmers. Body weight and body biometry were recorded on 82 adult males and 482 adult females. Due to various reasons, including non-identification of sheep in the farmers' flocks, culling of the lambs based on the decision of individual sheep owner etc., it was not possible to record the body weights of the same lamb at various specific ages. Therefore, body weights of 70 male and 89 female lambs, which belonged to various ages, were recorded. An age-weight relationship graph was plotted and regression equation fitted to get an estimated body weights of lambs at specific ages. The information on agro-climatic conditions of the region were collected from the published literature.

Distribution and number

Kenguri sheep prevails primarily in Koppal and Raichur districts of Karntaka state. While some animals of this breed, though in lower proportion of the

Fig 1. Distribution area of Kenguri Sheep

Table 1: Sheep Population (number in million)

Year	India	Karnataka state	Raichur district	Koppala district
1961	40.200	4.765	-	-
1966	42.015	4.748	-	-
1972	39.993	4.662	0.216	-
1977	40.907	4.536	0.262	-
1982	48.765	4.792	0.325	-
1987	45.703	4.727	0.364	-
1992	50.783	5.431	0.422	-
1997	56.361	8.003	0.379	0.192

Note: Before 1997, Koppla district was a part of the Raichur district. From livestock census of 1997 onward the livestock populations of the two districts are shown separately.

flock and in lesser pure form with respect to breed characteristic, are also found in the adjoining area of Bagalkot, Gulbarga and Bellary districts of the state. Kenguri sheep distribution area is bound by the rivers Krishna in north and Tungbhadra in south.

Sheep population of Raichur districts showed small fluctuations from 1961 through 1977, but thereafter registered an increase of about 5.9 per cent from 1977 through 1997 (Raichur and Koppal taken together). The increase in sheep population of Karnataka state from 1992 to 1997 was much pronounced as compared to the trend at national level and in the sheep population of Raichur district.

The average flock size was 87 comprising 4 adult males, 60 adult females and 23 young ones. However, the proportion of the young ones in the flock may vary widely depending upon the survey month when the observations are recorded. Majority of the Kenguri flocks could be categorized as medium sized, some as large sized and a few small sized; very small flocks were rare. Most of the Kenguri flocks in Koppal and Raichur districts were

purebred; the non-descript or other type of sheep in the flocks were below five per cent on an average. A few farmers tried to maintain Bellary sheep or breed the Kenguri ewes with a Bellary ram. Such farmers shifted back to Kenguri sheep very soon due to better growth of Kenguri lambs, poor adaptability of Bellary sheep in the red soil of the area and problem of shearing of Bellary sheep. There were only 1.8 % crossbred sheep in these districts as per 1997 livestock census.

Agro-climatic conditions

Breeding tract of Kenguri sheep falls in the Northern Dry Zone of Karnataka State. The climate is tropical with monsoon type rainfall distribution. The rainfall is scarce; average annual rainfall ranged from 633 to 807 mm. The minimum temperature reached 18.0 °C in winter and the maximum 39.6 °C in summer. The altitude is 465-786 metres from m.s.l. The soil is red sandy loam or red loam in major area and shallow black in limited area. The crops grown in the area comprised Sorghum (*Sorghum bicolor)*, Bajra (*Pennisitum typhodes*), Groundnut (*Arachis hypogea*), pulses, and oilseeds.

Socio-economic conditions of the farmers

Most of the sheep farmers were in low-income group. Average family size was 7 with equal number of males and females. Literacy rate was about 20%. However, the literacy percentage amongst the sheep graziers was very low. Sheep flocks are grazed by the owner or one of his family members. Female members play an important role in animal management. Besides sheep, the farmers also maintain cattle, buffaloes, goats, donkey, dogs and poultry. The dogs prevent the sheep from going astray besides the watch at night.

Management and housing

Flocks are taken out for grazing at about 8-9 o'clock in the morning and return in the evening at sunset. The sheep graze on crop stubbles, barren land and roadsides. In summer months, young lambs of less than three months of age are grazed in cooler hours of the day i.e. in the morning and evening. During hot hours i.e. from about 11 in the morning to 4 o'clock in the afternoon the lambs are housed in a shed. Flocks cover a distance of 8-12 km in a day for grazing. Kenguri sheep flocks are stationary and do not go on migration. Supplementary feeding is rare. Sheep are taken to water source two or three times a day. The sheep in the region utilize the poor grazing resources, yet contribute significantly to the farmers' income. Farmers vaccinate their sheep against haemorrgagic septicaemia, enterotoxaemia and sheep pox. Mortality rate was about 7% in adults and 8-10% in lambs. Acharya (1982) has reported higher mortality of 10 to 15% and 10 to 20% in adult and young stock respectively.

Fig 2. A sheep flock grazing in barren land

Fig 3. Housing of lambs during hot hours in summer

Animals are housed at night in a fenced enclosure with a shed in it, in most cases. The fence is prepared from bushes, other locally available material or irons wires whereas the shed is thatched with tree branches and crop byproducts. Sheep house is either a part of owner's dwelling or adjacent to it.

Breed characteristics

Kenguri is also known as Tenguri. Both the names are derived from the characteristic coat colour of the breed (Kemp=red + kuri= sheep; Teng=coconut, kuri= sheep). The sheep are large in size. Body coat is dark brown or coconut coloured. In most cases, there is a white spot on the forehead and sometimes on legs and other body parts also. Some of the Kenguri sheep have black belly and are known as 'JODKA'. Some of the farmers have preference for JODKA type and therefore are selected as breeding stock for propogation. A few sheep, known as 'MASAKA', had a mixture of brown and black color. Ears are medium long and drooping. Average ear length was 14.1±0.09 cm. Males are usually horned and females polled. About 85% males and 6% females were observed as horned. Average horn length was 33.6±0.97 cm in males and 10.2±0.52 cm in females. Tail was short and thin with an average length of 10.1±0.08 cm.

Fig 4. A Kenguri ram

Fig 5. A Kenguri ewe

Table 2: Body weight and biometry of adult Kenguri sheep

Body character	Adult Males		Adult Females	
	Average ± SE	Range	Average ± SE	Range
Body weight (kg)	52.6±0.86 (82)	32-72	35.9±0.24 (416)	23.5-57.5
Body length (cm)	74.7±0.45 (82)	65-84	67.3±0.15 (418)	59-77
Height at wither (cm)	81.5±0.43 (82)	70-92	72.6±0.14 (418)	64-85
Chest girth (cm)	89.4±0.54 (82)	76-99	78.4±0.20 (418)	60-92
Paunch girth (cm)	84.5±0.79 (82)	68-99	76.9±0.28 (418)	56-93
Ear length	15.2±0.14 (82)	11-18	15.3±0.07 (418)	8-20
Horn length	33.6±0.97 (69)	9-48	10.2±0.52 (25)	3-16
Tail length	12.0±0.22 (82)	8-17	10.1±0.08 (418)	5-16

Note: Within parentheses is number of observations

Genetic structure

Awareness for the importance of genetic resources has shoved studies on genetic diversity present across breeds within a species. At molecular level, microsatellites are the choice markers to characterize the genetic diversity among cattle, pig and sheep breeds (Buchanan *et al.*, 1994; MacHugh *et al.*, 1998; Saitbekova *et al.*, 1999; Diez-Tascon *et al.*, 2000; Li *et al.*, 2000; Arranz *et al.*1998., 2001; Barker *et al.*, 2001; Kim *et al.*, 2002). The available information to assess genetic variation and establish genetic relationships among the indigenous sheep breeds is limited (Sodhi *et al.*, 2003; Arora and Bhatia, 2004). Therefore, a study was undertaken to establish genetic variation in Kenguri sheep.

To assess the genetic structure of Kengiri sheep, genotyping with 20 microsatellite markers was carried out following the guidelines proposed under 'Measurement of Domestic Animal Diversity' programme (FAO, 1996). Blood samples (5-6 ml) were collected from 50 Kenguri animals taken at random from the breeding tract of the breed. Care was taken that the animals were genetically unrelated.

Genomic DNA was extracted from whole blood by standard phenol-chloroform extraction procedure. A panel of 20 microsatellite markers was selected for genetic characterization and revealing the genetic diversity in Kenguri sheep.

Table 3: List of microsatellite markers

Marker	Primer sequences (5' - 3')	Chromosome number
BM6506	GCACGTGGTAAAGAGATGGCAGCAACTTGAGCATGGCAC	1
BM757	TGGAAACAATGTAAACCTGGGTTGAGCCACCAAGGAACC	9
BM8125	CTCTATCTGTGGAAAAGGTGGGGGGGGTTAGACTTCAACATACG	17
BM827	GGGCTGGTCGTATGCTGAGGTTGGACTTGCTGAAGTGACC	3
HUJ616	TTCAAACTACACATTGACAGGGGGACCTTTGGCAATGGAAGG	13
ILSTS005	GGAAGCAATGAAATCTATAGCCTGTTCTGTGAGTTTGTAAGC	7
OarAE129	TCCAGTGTGTGAAAGACTAATCCAGGTAGATCAAGATATAGAATATTTTTCAACACC	5
OarCP34	GCTGAACAATGTGATATGTTCAGGGGGACAATACTGTCTTAGATGCTGC	3
OarFCB128	CAGCTGAGCAACTAAGACATACATGCGATTAAAGCATCTTCTCTTTATTTCCTCGC	2
OarFCB48	GAGTTAGTACAAGGATGACAAGAGGCACGACTCTAGAGGATCGCAAAGAACCAG	17
OarHH35	ATTGCATTCAGTATCTTTAACATCTGGCATGAAAATATAAAGAGAATGAACCACACGG	4
OarHH41	TCCACAGGCTTAAATCTATATAGCAACCCCAGCTAAAGATAAAAGATGATGTGGGAG	10
OarHH47	TTATTGACAAACTCTCTTCCTAACTCCACCGTAGTTATTTAAAAAAATATCATACCTCTTAAGG	18
OarHH64	CGTTCCCTCACTATGGAAAGTTATATATGCCACTCTATTGTAAGAATTTGAATGAGAGC	4
OarJMP29	GTATACACGTGGACACCGCTTTGTACGAAGTGGCAAGATTCAGAGGGGAAG	24
OarVH72	CTCTAGAGGATCTGGAATGCAAAGCTCGGCCTCTCAAGGGGCAAGAGCAGG	25
OMHC1	ATCTGGTGGGCTACAGTCCATGGCAATGCTTTCTAAATTCTGAGGAA	20
RM4	CAGCAAAATATCAGCAAACCTCCACCTGGGAAGGCCTTTA	15

Polymerase Chain Reaction (PCR) was carried out using PTC-100 thermocycler (MJ Research Inc., MA, USA). The PCR reaction of 25 ml contained 50-100 ng of template DNA, 200 mM each of dATP, dCTP, dGTP and dTTP, 50 mM of KCl, 1.5 mM of $MgCl_2$, 10 mM of Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.75 units of Taq DNA polymerase and 100 ng of each primer. After denaturing at 94 °C for one minute, the touchdown PCR programme was used as given below:

Table 4: Touchdown PCR programme

No of cycles	Temp.	Time	Temp.	Time
3 cycles	92 °C	1 min	60 °C	45 sec
3 cycles	92 °C	1 min	57 °C	45 sec
3 cycles	92 °C	1 min	54 °C	45 sec
3 cycles	92 °C	1 min	51 °C	45 sec
20 cycles	92 °C	1 min	48 °C	45 sec

The PCR products were resolved on 6% denaturing polyacrylamide gel. The resolved bands of DNA (alleles) were visualized by silver staining (Bassam et al, 1991). The genotypes were scored manually and allele size was calculated using INCHWORM programme. Genotype of each individual animal was recorded for each of the loci under consideration from the silver stained gels.

Various measurements of within breed genetic variations viz. number of alleles, allelic frequencies, effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e) were estimated using POPGENE software package (Yeh *et al.* 1999). Allelic frequencies were used to calculate polymorphic information content (PIC) following Botstein *et al.* (1980). The bottleneck hypothesis was investigated using BOTTLENECK programme (Cornuet and Luikart, 1996).

Various measure of genetic variation viz. observed and effective number of alleles, observed and expected heterozygosity, Polymorphic Information Content (PIC) value and Wright's (1978) fixation index (F_{IS}) for each of the microsatellte markers are given in Table 5. All the loci were found to be polymorphic and the observed number of alleles varied from 2 (BM6506) to 10 (OarHH35) with an overall mean of 6.55±0.20. A total of 131 alleles were detected across the 20 loci under analysis. The effective number of alleles was significantly lower than the observed number of alleles, which indicated larger deviation of allelic frequencies from the average values in the population. The variation in the allelic frequencies and the values of Shannon's Information index indicated the suitability of these loci to study the genetic structure of the population.

Nei's (1973) expected heterozygosity ranged from 0.44 (OarAE129) to 0.87 (OarHH35) with an overall mean of 0.72±0.02. The observed heterozygosity averaged 0.52±0.02 with a range from 0.10 (OarAE129) to 0.81 (OarJMP29). The average observed heterozygosity was less than the expected. Of the 20 loci, four and six loci based on Chi-square and G-square tests respectively showed significant ($p<0.05$) deviation from Hardy Weinberg equilibrium. The PIC values varied from 0.41 (Oar AE129) to 0.87(OarHH35) with an average of 0.70±0.03. Since microsatellites having PIC values higher than 0.5

are considered highly informative (Botstin *et al.* 1980), therefore, all the loci, with the exceptions of BM6506 and OarAE129, can be considered as very informative.

Table 5: Measure of genetic variation in Kenguri sheep

Locus	Sample Size	na*	ne*	I*	Obs_ Het	Exp_ Het*	Nei**	PIC	Fis
BM757	86	5	3.36	1.34	0.65	0.71	0.70	0.68	0.07
BM827	80	7	3.28	1.40	0.38	0.70	0.69	0.67	0.46
BM6506	92	2	1.99	0.69	0.43	0.50	0.50	0.43	0.12
BM6526	80	8	6.39	1.93	0.65	0.85	0.84	0.83	0.23
BM8125	88	7	3.28	1.40	0.32	0.70	0.69	0.67	0.54
HUJ616	88	5	3.18	1.33	0.16	0.69	0.69	0.66	0.77
ILSTS005	94	6	4.77	1.64	0.70	0.80	0.79	0.77	0.11
OarAE129	82	4	1.77	0.79	0.10	0.44	0.44	0.41	0.78
OarCP34	64	5	4.12	1.50	0.50	0.77	0.76	0.74	0.34
OarFCB48	76	8	6.62	1.99	0.66	0.86	0.85	0.84	0.23
OarFCB128	74	7	3.83	1.55	0.65	0.75	0.74	0.72	0.12
OarHH35	82	10	7.82	2.15	0.59	0.88	0.87	0.87	0.33
OarHH41	66	9	5.42	1.93	0.73	0.83	0.82	0.81	0.11
OarHH47	72	8	5.99	1.89	0.47	0.84	0.83	0.82	0.43
OarHH64	92	7	2.42	1.24	0.48	0.59	0.59	0.57	0.19
OarJMP8	88	6	3.30	1.43	0.61	0.70	0.70	0.68	0.12
OarJMP29	94	6	3.51	1.42	0.81	0.72	0.72	0.69	-0.13
OarVH72	86	7	4.62	1.68	0.49	0.79	0.78	0.77	0.38
OMHC1	88	8	3.92	1.65	0.45	0.75	0.74	0.73	0.39
RM4	92	6	3.27	1.42	0.50	0.70	0.69	0.67	0.28
Mean	83	6.55	4.14	1.52	0.52	0.73	0.72	0.70	
SE		**0.20**	**0.18**	**0.08**	**0.02**	**0.01**	**0.02**	**0.03**	

* na :Observed number of alleles
* ne :effective number of alleles (Kimura and Crow, 1964)
* I :Shannon's Information index (Lewontin, 1972)
** :Nei's (1973) expected heterozygosity
PIC :polymorphic information content
Fis :is the Wright's (1978) fixation index, a measure of heterozygote deficiency or excess

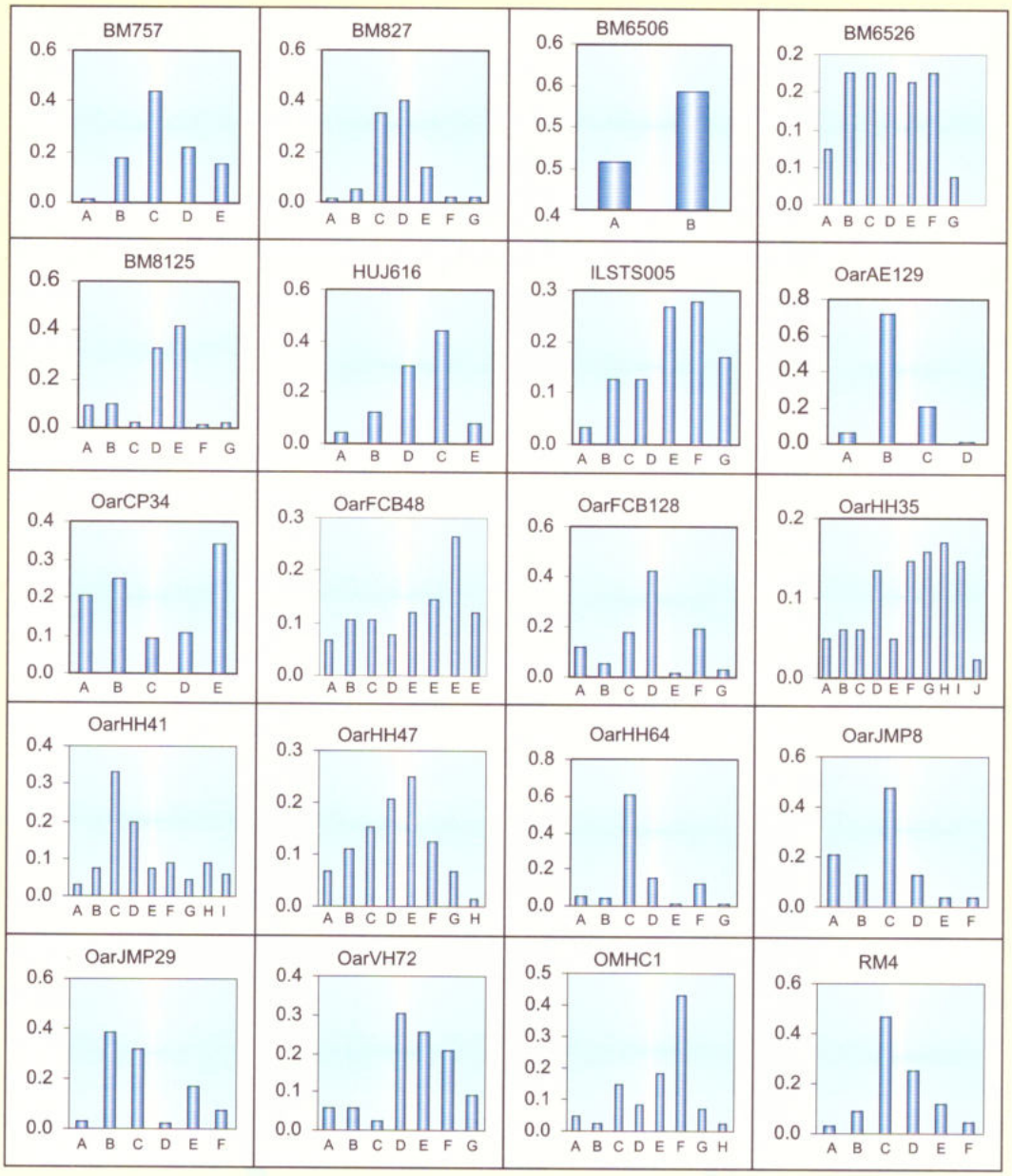

Fig 6. Allelic frequencies at microsatellite loci in Kenguri sheep

Average heterozygosity and other parameters computed are comparable with other Indian sheep breeds viz. Muzzafarnagri 0.652 (Arora and Bhatia, 2004) and Garole 0.603 (Sodhi *et al.*, 2003). The average genetic variation reported for Swiss sheep breed Mouflon 0.450 (Stahlberger-Satbekova *et al.*, 2001), Northern Spanish sheep-Latxa 0.661, Black-faced Latxa 0.594, Rubin del Molar 0.600, Churra 0.661, Xalda 0.572 (Alvarez *et al.*, 2004) are also of the similar range. However, the observed genetic variation in Kenguri sheep is slightly lower than that of Spanish sheep breed 0.771 and Awassi sheep from Turkey 0.750 (Arranz *et al.*, 1998).

Wright's (1978) fixation index (F_{IS}) revealed the population to be heterozygote deficit at 19 loci and heterozygote excess at one locus that is OarJMP29. The F_{IS} values ranged from -0.13 to 0.78 for various loci. The heterozygote deficiency in the population may be explained on account of mating the ewes in a farmer's flock by the same ram for a long period. Indiscriminate mating and use of the same ram for breeding within a flock might have led to consanguineous mating, which in turn might have resulted into heterozygote deficiency. Since nineteen of the twenty loci were heterozygote deficit, therefore we might consider this as the principal cause of heterozygote deficiency. Besides, close linkage of some of these microsatellite loci with the economic traits for which selection is being practiced might have also contributed to heterozygote deficiency in the population

Analysis of the allelic frequencies considering two loci together (Weir, 1979) revealed significant ($P < 0.05$) linkage disequilibrium in 20 combinations in the population. Smouse's multilocus analysis for single population revealed non-random association of alleles at different loci and departures from multiple-locus panmixia in the population. This could be due to population subdivision or agglomeration within the population, genetic drift or association of the microsatellite markers with the fitness/ production traits, which are under selection.

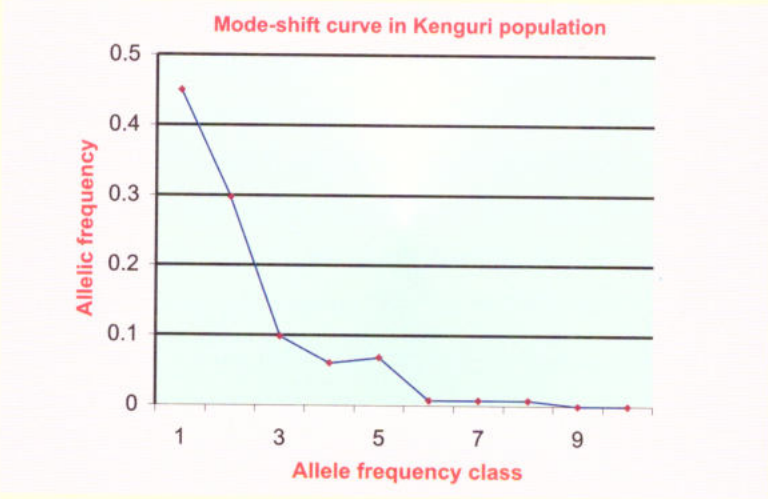

Fig 7. Mode shift curve in Kenguri population

The population was investigated for recent bottleneck by using BOTTLENECK programme (Cornuet and Luikart, 1996). This programme works on the principal that any population that has experienced a recent reduction in their effective population size exhibit a correlative reduction of the allele numbers (k) and gene diversity (He, or Hary-Weinberg heterozygosity) at polymorphic loci. But the allele numbers is reduced faster than the gene diversity. Thus, in a recently bottlenecked population, the observed gene diversity (Heterozygosity) is higher than the expected equilibrium gene diversity (Heq), which is computed from the observed number of alleles (k), under the assumption of population at equilibrium (Luikart *et al.* 1998). In a population at mutation-drift equilibrium there is approximately an equal probability that a locus shows gene diversity excess or gene diversity deficiency. *If* a population exhibits a significant number of loci with gene diversity excess, then the population is considered to have undergone recent bottleneck.

The population was anlysed for recent bottleneck under Two-phased model of mutation (TPM) because most microsatellite data sets are considered to better fit this model (TPM) than Stepwise Mutation Model or Infinite Allele Model. The difference between the expected number of loci with heterozygosity (11.73) and the observed number of loci with heterozygosity excess (16) was statistically significant under the Sign test. Standardized Differences test and Wilcoxon test under the assumption of TPM revealed that the population has not suffered recent bottleneck because the observed heterozygosity was not in excess of expected heterozygosity. However qualitative graphical method of Luikart and Cornuet (1996) was also used to visualize the allele frequency spectra (Fig. 7). The microsatellite alleles were classified in 10 frequency classes. The L-shaped frequency spectra revealed that the population had not undergone bottleneck at least in the recent past.

Reproduction

Most of the flocks were purebred. Kenguri rams are selected on the basis of body size and conformation. Body length, height at wither and coat color are given due weightage in the order of priority; white patch on forehead is preferred. September to November is the main lambing season and April to May the minor. Lambing rate was about 82% with an average lambing interval of about 14 months. Litter size

Fig 8. Pregnant ewes grazing in the field

is single with rare case of twinning. A ewe produced about 4-5 lambs in its lifetime.

Body weight of lambs

The body weights of male and female lambs collected from the farmers' flocks were plotted against the age of the lambs to get an estimate of body

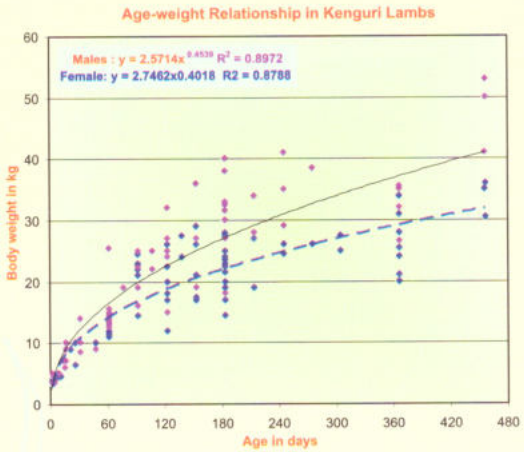

Fig 9. Age-weight relationship in Kenguri lambs

weight at various important ages. A power curve gave the best fit. The estimated body weights of the male and female lambs by using these equations are given in Table 6.

Table 6: Estimated body weights of Kenguri lambs

Age	Body weight (kg)	
	Male lambs	Female lambs
Birth	2.57	2.75
2-month	16.6	14.3
3 months	19.9	16.8
6 months	27.4	22.3
12 months	37.4	29.4

Production

In most sheep breeds of southern and eastern regions of India, there is little income from wool. As such major income is from sale of male lambs and other categories of live animals. Kenguri sheep is maintained primarily for mutton. In addition to this, there is some income from milk and dung. Body is covered with coconut coloured thick shiny hair, which is never shorn. Therefore, there is no income from wool in this sheep breed. Lactating ewes, whose lambs have died or sold off, are milked for home consumption or occasionally for sale of milk. In some cases, extra milk is stripped out after feeding the lambs. Lactation length was about 4 months and a ewe produced 300-500 ml milk per day during peak months.

Fig 10. A typical Kenguri flock

Surplus male lambs are sold at 2-5 months of age for Rs 800-1000/- depending upon the condition and body weight. The ewe lambs are kept as replacement. The old ewes and rams are sold at Rs 800-1100 and Rs 1500-2500 respectively. The sheep are generally sold in a weekly market called 'SANDY'. Good rams are sold to needy sheep farmers.

Fig 11. Marketed male lambs

Conservation and improvement

The population size, trend over the years and the practice of selective breeding in Kenguri flocks suggested a normal status of the breed without any endangerment of extinction. Integration of the sheep in the agro-ecology, availability of feed and fodder resources in the area, and promising growth rate of the Kenguri lambs make sheep rearing a remunerative and sustainable means of livelihood and employment for farmers and their family members in the area. Farmers need to be educated regarding the ill effects of the inbreeding. To begin with, a Kenguri Sheep improvement programme may be undertaken to plan and demonstrate the exchange of the rams between the elite flocks on one hand and at the same time to provide good breeding males to resource poor and needy farmers for genetic improvement of their flocks.

Acknowledgement

The Director, National Bureau of Animal Genetic Resources, Karnal provided necessary facilities for the study on characterization and evaluation of Kenguri sheep. The Director, State Animal Husbandry and Veterinary Services, Karnataka and other officers/officials provided necessary help and support during the survey in farmers' flocks. The authors are thankful to all of them.

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Published by : **Director, NBAGR**

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