



Characterization of a new potential goat breed (*Palamu*) from Jharkhand, India

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ABSTRACT

Palamu goat forms the part and parcel of the lives of the farmers and tribes of Jharkhand. Palamu goat also known as *Medini* is named after its distribution area (Palamu, Latehar and Garhwa) in Jharkhand. This small size goat is reared in Jharkhand since time immemorial. Average flock size is 5.36 ± 0.19 and varies from 1 to 31. Twinning is common except in first kidding. Head profile is convex, ears are pendulous, and horns are straight with backward and upward orientation. Muzzle, eyelids and hooves are black, body is cylindrical, udder is small and pendulous and teats are conical shaped. Age at first mating in males is 8.32 ± 0.86 (months) that vary between 7.2–9.2 months. Corresponding values for female are 7.22 ± 1.35 months varying between 6.7–7.7 months. Diversity status of the population was explored using 25 microsatellite markers. A total of 190 alleles were detected and sufficient polymorphism was evident from the allele frequency data. ILSTS82 showed the highest number of observed alleles per locus (20) while RM4 and ILSTS05 showed the lowest (4) with 9.14 ± 2.0 as mean number of alleles. Expected number of alleles varied from 1.49 (ILSTS065) to 7.55 (ILSTS30) with the mean value of 4.15 ± 0.91 . Palamu goat had substantial genetic variation based on its gene diversity in addition to the average number of alleles per locus. The observed and expected heterozygosity values were 0.64 ± 0.14 and 0.69 ± 0.15 , respectively. Observed heterozygosity was lower than expected showing a departure from Hardy-Weinberg Equilibrium (HWE) and possibility of inbreeding. Population has heterozygote deficiency to the tune of 9% (F_{IS} value=0.09). Population did not suffer from recent genetic bottleneck (last 40–80 generations). The results suggest existence of a distinct goat population harboring sufficient genetic variation for scientific management.

Keywords: Characterization, Genetic diversity, Jharkhand, Microsatellite, Morphometric traits, Palamu goat

Goats have the widest ecological range among all species of farm animals, and have been poor peoples' most reliable livelihood resource since their domestication during Neolithic period about 10 millennia ago. Goat plays a significant role in providing supplementary income and livelihood to millions of resource poor farmers and landless labourers of rural India (Kumar *et al.* 2020). Goat rearing ensures self-employment and acts as a cushion in distress situations like drought and famine. In the present scenario of changing agro-climatic conditions, this small ruminant farm animal has tremendous potential for rural and urban prosperity. More often goats are reared for production of meat, but they also serve as ready source for milk to meet the family requirement (Sharma *et al.* 2020).

Total number of Indian livestock is showing an increase in the recent past as it has risen to 535.78 million in 2019 from the last livestock census figures of 512 million in 2012 (20th Livestock Census 2019). This increment is driven by

a sharp increase in the number of small ruminants such as sheep and goats, which is nearly 95% of the total livestock increase. As per Livestock Census 2019, there are 148.88 million goats in the country and has registered an increase of 10.14%. Ten states in India account for 80% of the country's goat population. Jharkhand is ranked 7th with 9.12 million goat which accounts for 6.1% of total Indian goat population. Goat population has also registered a significant increase in Jharkhand (38.6%). However, all the goats in Jharkhand are considered as non-descript and no population has been registered as a breed. Non-descript population refers to the cross-bred populations, populations which are mixture of different breeds or the populations which have not yet been studied or described. The scientifically planned documentation of such populations is essentially required. The complete characterization (phenotypic and genetic) of these populations may indicate the distinctness of some or all of these populations. After confirming the distinctness, the population can be registered as a breed and the useful characteristics of the breed can be utilized for upgrading other non-descript populations. Thus the emphasis at present should be to describe, characterize and document lesser known populations in the country so that the proportion of

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non-descript population is considerably transformed in to defined breeds. This will be the first step towards planning the organized breeding program for their genetic improvement, conservation strategies and sustainable utilization (Mishra *et al.* 2015). In the last few years, goat characterization and registration gained momentum in the country. As a result, 34 goat breeds have been registered by the ICAR gazette notification (www.icar.org.in). Registered goat breeds accounts for 41% of indigenous goat population. However, 59% of total goats are still not categorized.

Goat rearing is an essential part of the lives of the resource poor farmers especially the women in Jharkhand and of its rural economy. Goats are also an important part of the society and culture, especially for the tribal farmers and are the preferred meat during marriages and festivals despite its ever rising prices. Thus, research was carried out to explore and validate any uniform goat population existing in Jharkhand that can be registered as a breed. Phenotypic characterization under field conditions is helpful in defining a population on the basis of morphological characteristics. In addition, an investigation for genetic variation within population was done to help in evaluate how likely various factors are responsible for affecting its genetic variability so that suitable measures may be undertaken for maintaining variability and purity of the population.

MATERIALS AND METHODS

Phenotypic characterization: Preliminary survey indicated that breeding tract runs across three districts, viz. Palamu, Latehar and Garhwa located between latitude 23° 70' to 24° 46' N and longitude 83° 52' to 85° 22' E in Jharkhand. The state receives annual rainfall of 1200–1600 mm and the climate ranges from dry semi humid to humid semi arid types. Close to 78% of its population in rural areas, with about a quarter of the population belonging to the tribal community. Paddy is major crop being produced and most of the cultivation in the state is dependent on rainfall. Information on the body biometric characteristics, viz. body length from shoulder to pin bone, chest girth, height at wither, paunch girth, face length, ear length, tail length and horn length, and qualitative confirmation attributes and body weights of randomly selected 167 animals (both male and female) were recorded. The information on feed, management, breeding practices and reproductive performance in the breeding tract was collected through a structured questionnaire. Data on body biometric attributes and body weights were analyzed using SPSS 11.5 for windows.

Blood sample collection and molecular characterization: One or two animals were selected per flock per farmer. Farmers were interviewed in detail about relatedness of animals before sampling so as to avoid relationship among the animals. Blood was collected from jugular vein in 10 ml vacutainer tubes having EDTA (Ethylene diamine tetra acetic acid) as anticoagulant.

Samples were brought at 4°C in the laboratory and stored at –20°C until DNA extraction. Isolation of Genomic DNA was performed using phenol-chloroform extraction method. The integrity and quantity of DNA was assessed through 1% agarose gel by direct comparison with a standard marker as well as spectrophotometrically (Nanodrop spectrophotometer). Twenty five FAO (<http://dad.fao.org/en/refer/library/guideline/marker.pdf>) and ISAG (International Society for Animal Genetics) recommended microsatellite markers for goat were selected for the diversity analysis. These were highly polymorphic markers spread across the genome. Forward primer of each marker was 5' labeled with a fluorescent dye (FAM, VIC, NED and PET). PCR amplification was performed in 10 µl reaction volume. Reaction mixture consisted of 10–20 ng of DNA, 0.2 µM of each primer and PCR master mix consisting of 0.2 mM of each dNTP and 2 mM of MgCl₂. A negative control, consisting of all the reaction components, except for the template DNA, was also included to detect any possible contamination. Touchdown protocol was run which included initial denaturation of 95°C for 1 min; amplification cycle with denaturation at 95°C for 45 sec, 60–51°C with decrease of 3°C every third cycle for 1 min, 72°C for 45 sec and 20 cycles of denaturation 95°C for 45 sec, amplification at 48°C for 1 min, extension at 72°C for 45 sec followed by final extension step at 72°C for 5 min. Two loci (ILSTS049 and OarAE129) were amplified with specific temperature protocol which consisted of initial denaturation of 95°C for 1 min; 32 cycles of 95°C for 30 sec, specific annealing temperature (58 and 60°C, respectively) for 45 sec, 72°C for 45 sec and final extension step at 72°C for 10 min. The amplified products were electrophoresed on a 1.8% agarose gel treated with ethidium bromide (0.5 mg/ml) for visualization of DNA bands under ultraviolet light. PCR products were multiplexed and genotyping was carried out on an automated DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was done using GeneMapper software v3.7.

Statistical analysis: Basic genetic parameters including allele frequencies, observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population were calculated by analyzing genotype data with GenAlEx v6.5 software (Peakall and Smouse 2012). Bottleneck v1.2.02 (<http://www.ensam.inra.fr/URLB>) software was used to test bottleneck events in the population by 2 approaches. The first approach consisted of three heterozygosity tests developed by Cornuet and Luikart (1996): (i) Sign test, (ii) Standardized differences test, and (iii) Wilcoxon sign-rank test. The probability distribution was established using 1000 simulations under 3 models–Infinite allele model (IAM), step-wise mutation model (SMM) and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart *et al.* (1998).

RESULTS AND DISCUSSION

Distribution and description: Goat rearing is practiced by a large number of farmers in the Jharkhand and the large forest cover ensures availability of feed and fodder for most part of the year. About 80% of the rural population is dependent on agriculture and livestock for sustaining their livelihood. Palamu goat is named after region of its distribution. It is also referred to as Medini goat, named after ruler of the kingdom of Malwa, 'Medini Rai' in the early decades of the 1500s. Medini Rai, according to contemporary sources, was considered to be guardian of native livestock breeds. Palamu goat is distributed in more than 12,000 square kilometre area encompassing Palamu, Latehar and Garhwa and adjoining districts of Jharkhand. Total goat population of breeding tract was estimated to be 0.57 million. Major communities rearing these goats in the breeding tract were Bhuiyan, Yadavs and tribal communities.

Animals of this breed are small in size and are of black color with hairy and shiny coat. Hairs are distributed all over the body (Fig. 1). Muzzle, eyelids and hooves are



Fig. 1. Palamu goat depicting morphometric characteristics.

black. Body is cylindrical with straight forehead. Head profile is convex and ears are pendulous. Beard is absent and tail is short. Majority of animals have horns. Wattles were absent. Horns were straight. Orientation was backward and upward. The average body measurement of male and female goat is presented in Table 1. Body weights at different stages of growth are presented in Table 2. Mean birth weight of male kids was higher as compared to female kids. Average weight of adult male was 29.89 ± 0.72 kg whereas, female was comparatively lighter at 22.71 ± 0.41 kg. Goats have small pendulous udder with conical teats.

Management and breeding practices: Extensive system of management is largely practiced due to availability of abundant forest cover. Most of the farmers prefer grazing throughout the year except during one to two months of monsoon season when regular grazing is not possible. During rainy season, goats are fed with leaves and other greens at home by the farmers without concentrate ration. Diarrhea and dehydration was reported during monsoon. Use of anti-parasitic and deworming as well as vaccination is not common due to the poor awareness among the farmers.

Average herd size was 5.36 ± 0.19 . Herd size varied across households and districts from as low as a single animal to as high as 31 goats. A typical flock on an average was constituted by 14.78% adult female, 13.52% adult male, 20.16% male kids and 51.54% female kids. Housing was provided only during night time. Majority of goats were housed in open kutchcha houses having biological boundaries.

Performance: Goats were good breeders showing first oestrous at an average age of 6.99 ± 1.33 months and had a range of 6.5–7.5 months. Their age at first mating was 7.22 ± 1.35 months which varied between 6.67–7.67 months. Males also showed sexual maturity at an age of around 8

Table 1. The average ($\pm$ SE) biometrical characteristics (cm) of Palamu goat

Body measurement	Male			Female		
	Average	Range	N	Average	Range	N
Chest-girth	72.16 ± 1.18	64.50–78.90	16	66.86 ± 0.48	61.40–72.60	51
Body length	60.36 ± 1.01	54.10–67.40	16	53.77 ± 0.49	48.50–59.60	51
Height at withers	62.77 ± 0.54	59.50–65.10	16	51.53 ± 0.53	45.70–58.50	51
Horn length	9.25 ± 1.34	6.00–14.00	16	7.72 ± 0.84	5.00–11.00	51
Tail length	16.74 ± 0.94	12.00–22.00	16	14.84 ± 1.78	10.00–21.00	51

Table 2. The average ($\pm$ SE) body weights (kg) of Palamu goat

Weight at	Male			Female		
	Average	Range	N	Average	Range	N
Birth	1.29 ± 0.09	0.80–1.70	12	1.11 ± 0.08	0.80–1.50	9
Weaning / 3 month	5.80 ± 0.34	3.90–8.30	18	5.17 ± 0.19	4.40–6.10	15
6 months	10.66 ± 0.37	8.40–12.90	16	8.84 ± 0.28	6.50–11.30	28
9 months	14.56 ± 0.45	12.80–16.40	10	12.01 ± 0.31	9.50–14.20	23
1 year	17.77 ± 0.42	16.20–20.00	12	15.87 ± 0.23	13.70–18.40	41
6 Teeth (adult)	29.89 ± 0.72	25.70–33.80	16	22.71 ± 0.41	18.60–27.70	51

months. Average age at first mating was 8.32 ± 0.86 months with a range of 7.2–9.2 months. Oestrous cycle in females was of 20.55 ± 0.25 days varying between 18–25 days. Mean values for age at first kidding, gestation length, kidding interval in months was 12.15 ± 1.21 (11.66–12.60), 4.95 ± 0.30 (4.83–5.07) and 7.69 ± 1.31 (7.17–8.17), respectively. Service period varied between 41–119 days with an average of 80.94 ± 3.28 days. Castration of males is a common practice. Majority of the farmers reported the litter size to vary between one and three. Twinning is common but first kidding was single. Lifetime number of kiddings was 8.63 ± 0.24 which varied from 5–12 with an average litter size of 1.35 ± 0.06 .

Dairy performance was recorded on 45 does. Mean lactation milk yield was 18.09 kg that varied from 10.04 to 45.9 kg. The lactation length was observed to be between 62–95 days with an average of 79.44 days. Mean daily milk yield was only 227.3 g with a range of 119–540 g. Fat, SNF, protein and lactose percentage in Palamu goat milk was on an average 6.97 (5.51–8.89), 9.4 (8.1–10.4), 3.4 (2.9–3.8) and 5.14 (4.4–5.7), respectively. Carcass characteristics are given in Table 3.

Palamu goat resembles with the famous registered) Black Bengal goat breed of India (INDIA_GOAT_2100_BLACKBENGAL_06004), in appearance. Black Bengal goats are widely distributed throughout West Bengal and adjoining neighboring states including Jharkhand. However, a perusal of body measurement revealed the difference among the two populations. Palamu goats are taller than the Black Bengal goat (Tudu *et al.* 2015). Average body weight of Black Bengal male is 13.46 ± 0.048 kg, whereas Palamu male were found to have mean body weight of 17.77 ± 0.42 kg at 12 months of age (Table 2). Body conformity is also distinct among the two, as Palamu goats are cylindrical in shape, whereas, Black Bengal goats look triangular from the side, with a heavier posterior region. In addition, Black Bengal goats have better fecundity, thus higher kidding rate than the Palamu goat.

These goats are not recognized as a registered breed and hence no specific breeding policy is in place for them. On the contrary, breeding policy of Jharkhand states that local goats shall be improved by crossbreeding/ upgrading with Beetal, Barberi, Sirohi and Jakhrana bucks. Local village bucks were used indiscriminately for breeding of the females resulting in loss of population uniformity.

Genetic characterization: Twenty three microsatellite (SSR) markers amplified successfully with DNA samples of Palamu goat. Less than 4 alleles were detected across

OarJMP29 (2) and OarFCB304 (1) and thus were excluded from further analysis. A total of 190 alleles were observed. Diversity estimates of Palamu goat population are given in Table 4. High allelic diversity was observed in Palamu goat population as ILSTS82 locus showed as high as twenty alleles. RM4 and ILSTS05 depicted the lowest (4) alleles among the selected loci. High value was observed for mean number of alleles per locus being 9.14 ± 2.0 . Effective number of alleles varied from 1.49 (ILSTS065) to 7.55 (ILSTS30) with the mean value of 4.15 ± 0.91 . Lesser values of effective number of alleles as compared to observed number of alleles in the population reflected towards the existence of several alleles at low frequency (Sharma *et al.* 2015).

Higher genetic variation observed in Palamu goats must be one of the factors contributing towards their adaptability. Palamu goat population harbors higher genetic variation than the recognized goat breed of the neighboring area, Black Bengal (8.53 ± 0.26 ; Vijn *et al.* 2010). Similarly, it was higher in comparison to other Indian goat breeds such as Mahboobnagar (8.8 ± 0.55 ; Raghavendra *et al.* 2017), Bidri (8.48 ± 0.88) and Nandidurga (8.22 ± 0.66 ; Tania *et al.* 2018). Much higher allelic diversity has been reported in Changthangi goat of Himalayan region (10.4 ± 3.91) (Mishra *et al.* 2010).

Observed heterozygosity in Palamu goat (Table 4) was less than the expected heterozygosity, suggesting deviation from occurrence of random mating among its individuals. The observed and expected heterozygosity ranged from 0.2 (ILSTS065) to 0.91 (OMHC1) and 0.33 (ILSTS065) to 0.87 (ILSTS30) with a mean of 0.64 ± 0.14 and 0.69 ± 0.15 , respectively. Mean observed heterozygosity values similar to that of Palamu goat were observed in Black Bengal (0.69; Vijn *et al.* 2010), Nandidurga (0.60; Tania *et al.* 2018) and Mahboobnagar (0.69; Raghavendra *et al.* 2017) goats. On the other hand, higher values were noticed in Osmanabadi (0.71; Bhat *et al.* 2013), Sanagamneri (0.73; Nath *et al.* 2014), Berari (0.79; Kharkar *et al.* 2015) and Chegu (0.80) and Gaddi (0.75) goat of Himachal Pradesh (Singh *et al.* 2015). Much lower values have been observed in the goat populations of North Eastern Hill (NEH) region such as Assam hill goat (0.48; Zaman *et al.* 2013) and Sumi-Ne goat of Nagaland (0.49; Verma *et al.* 2019).

A moderate positive value of F_{IS} (0.09 ± 0.02) indicated towards heterozygote deficiency in the population which may be contributed to the inbreeding. In fact, twelve loci deviated from the Hardy Weinberg equilibrium supporting occurrence of non random mating. However, 8.8% deficit

Table 3. Carcass characteristics of Palamu goat meat

Carcass characters	Male			Female		
	Average	Range	N	Average	Range	N
Age at slaughter (days)	290.00	210–365	10	—	—	—
Weight at slaughter (kg)	16.64	14.05–19.12	10	19.11	17.22–21.15	9
Dressing % (hot)	47.08	45.77–48.52	10	44.15	41.06–47.97	9

Table 4. Diversity estimates across microsatellite loci in Palamu goat

Locus	N	Na	Ne	I	Ho	He	uHe	F
ETH225	42.00	6.00	4.14	1.56	0.67	0.76	0.77	0.12
ILSTS044	37.00	9.00	2.32	1.31	0.46	0.57	0.58	0.19
ILSTS08	46.00	7.00	2.26	1.16	0.59	0.56	0.56	-0.05
OarHH64	45.00	8.00	5.10	1.83	0.69	0.80	0.81	0.14
ILSTS059	40.00	8.00	2.72	1.33	0.48	0.63	0.64	0.25
ILSTS065	45.00	5.00	1.49	0.70	0.20	0.33	0.33	0.39
OMHC1	42.00	11.00	7.20	2.13	0.91	0.86	0.87	-0.05
ILSTS033	44.00	12.00	2.61	1.54	0.68	0.62	0.62	-0.11
OarE129	44.00	9.00	4.88	1.85	0.84	0.80	0.80	-0.06
OarFCB48	45.00	10.00	6.61	2.03	0.84	0.85	0.86	0.01
ILSTS05	44.00	4.00	1.73	0.79	0.43	0.42	0.43	-0.03
ILSTS019	44.00	9.00	4.88	1.82	0.57	0.80	0.80	0.29
ILSTS058	45.00	12.00	3.09	1.68	0.56	0.68	0.68	0.18
ILSTS087	45.00	13.00	6.98	2.17	0.82	0.86	0.87	0.04
ILSTS049	43.00	7.00	3.99	1.55	0.74	0.75	0.76	0.01
ILSTS29	43.00	8.00	2.86	1.42	0.51	0.65	0.66	0.21
ILSTS30	44.00	11.00	7.55	2.17	0.68	0.87	0.88	0.21
ILSTS34	43.00	8.00	1.74	0.93	0.42	0.43	0.43	0.02
ILSTS22	46.00	11.00	4.84	1.83	0.87	0.79	0.80	-0.10
ILSTS82	46.00	20.00	7.17	2.36	0.94	0.86	0.87	-0.09
RM4	43.00	4.00	3.01	1.23	0.49	0.67	0.68	0.27
Mean	43.62	9.14	4.15	1.59	0.64	0.69	0.70	0.09
SE	9.52	2.00	0.91	0.35	0.14	0.15	0.15	0.02

Na, No. of different alleles; Ne, No. of effective alleles = $1/(\sum \pi^2)$; I, Shannon's information index = $-\sum [\pi \times \ln(\pi)]$; Ho, Observed heterozygosity = No. of Hets/N; He, Expected heterozygosity = $1 - \sum \pi^2$; F, Fixation index = $(He - Ho)/He = 1 - (Ho/He)$, where π is the frequency of the i^{th} allele for the population and $\sum \pi^2$ is the sum of the squared population allele frequencies.

of heterozygotes was comparatively less as compared to heterozygote deficiency reported in some other Indian goat breeds, viz. Changthangi (17.7%; Mishra *et al.* 2010) and Chegu (11.2; Vijh *et al.* 2010), Sikkim Singharey (22.5%; Shrivastava *et al.* 2017), Sumi-Ne (25.8%; Verma *et al.* 2019), Bidri (13.6%) and Nandidurga (13.7%) (Tantia *et al.* 2018).

Bottleneck assessment: Palamu population has not undergone any recent reduction in population size (Table 5). Heterozygosity excess was not significantly ($P > 0.05$) lower as per all the three tests under IAM and for all the three models under Wilcoxon test. Heterozygosity excess was significantly less ($P > 0.05$) for Sign test under SMM and for Standardized differences test under TPM and SMM only.

Table 5. Population bottleneck analysis for Palamu goat

Model used		IAM	TPM	SMM
Sign test (No. of loci with heterozygosity excess)	Exp	12.88	12.90	12.86
	Obs	11	10	6*
	P value	0.2724	0.1488	0.0031
Standardized differences test	T2 value	0.124	-4.268*	-11.609*
	P value	0.4506	0.00001	0.0000
Wilcoxon test (one tail for H excess)	P value	0.4368	0.9631	0.9996

*Null hypothesis that population is in under mutation-drift equilibrium is rejected.

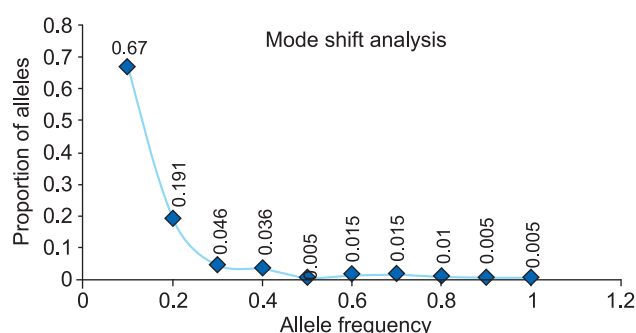


Fig. 2. Graphic representation of proportion of alleles and their distribution in Palamu goat population.

Graphical representation utilizing allelic class and proportion of alleles also showed a normal 'L'-shaped distribution (Fig. 2). Abundance of low frequency ($P < 0.10$) alleles ruled out chances of bottleneck event in the past evolutionary history of Palamu goat population.

Goat rearing for farmers or tribes of Jharkhand is a way of life rather than a financial enterprise. It is being carried out since time immemorial using traditional methods, without any intensive farming enterprise to increase profit generation. Goats are an asset to them and are considered to be a source of income in times of their need that varies from agriculture inputs, medical emergencies to social occasions like marriage. It may be concluded that indigenous goat in Jharkhand showed uniformity in physical

and morphometric characteristics and contributes significantly in the economy of the goat keepers. They had good potential for meat production and there is a need to develop genetic improvement programs to enhance the productivity. For achieving this target, Palamu should first be registered as a breed so that they get incorporated in the breeding policy. Moreover, microsatellite analysis identified plenty of genetic variation existing in Palamu goat population for their management, breeding, improvement and conservation.

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