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Molecular characterization, body parameters and management practices of Purnea cattle

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ABSTRACT

The present study is our contribution to the ongoing national endeavor of characterizing indigenous livestock genetic resources. The phenotypic parameters, management practices and genetic variability status of Purnea cattle were evaluated. A survey was conducted in different villages of Purnia commissionary of Bihar. These animals were kept under zero or very low input system of management. The animals were of small size and compact body. The coat colour of Purnea cattle ranged from light to deep red with few grayish white animals. The body length, chest girth, height at withers, paunch girth, face length, tail length with and without switch, horn length and ear length were measured in 205 animals belonging to different age/ sex groups. Purnea is the smallest cattle in Bihar based on morphometric measurements. The head profile was straight in females and slightly budged in males. The colour of eyelid, muzzle and tail switch were mostly black. The horns were short, set in a crescent shape and point forward. The udders were small in size and bowl in shape. The age at sexual maturity in males was about 2.5 to 3.0 years and age at first calving of cows was between 3 and 4 years of age. The average daily milk yield of cows was 0.5 to 2.0 kg. Genotyping was done with 23 bovine microsatellite markers as suggested by International Society for Animal Genetics (ISAG) and FAO (DAD-IS) on automated sequencer. A total of 204 alleles were observed. The mean number of observed alleles (No) and effective alleles (Ne) were 8.88±0.7 and 4.18±0.38 respectively. The average observed heterozygosity (Ho, 0.69±0.04) was less than the expected heterozygosity (He, 0.71±0.03). The f-estimates indicated 4.3% heterozygote deficit in the Purnea population. This obviously signifies that this breed retains considerable intensity of genetic variability and low level of inbreeding, notwithstanding its declining population in the breeding region. Investigation on present status of Purnea population based on physical and molecular characteristics demands its recognition as thirty-fifth breed of indigenous cattle.

Key words: Bihar, Cattle, Genetic variability, Indian cattle, Microsatellite, Purnea cattle

India is a reservoir of cattle diversity. In addition to 34 recognized breeds of cattle, various cattle populations exist in India, which have not yet been documented. Effort is in progress to characterize these populations at the phenotypic and molecular levels, and to document their production, environments, utilizations and status. Local cattle are important to preserve as they are well adapted to climate, food supply and other local environmental factors, which often shows in their superior robustness. The use of new breeding techniques, introduction of highly productive breeds, demographic pressure and support of industrial agriculture is contributing to the loss of valuable traits or decrease in number of animals of local breeds. Diverse attributes of a population are effective in its characterization, taking account of phenotypic traits (monogenic and polygenic), reproduction, geographic distribution, origin and habitat. The genetic characterization of populations, breeds and species allows the evaluation of genetic variability, a fundamental element in working out breeding strategies and genetic conservation plans. Molecular markers have been comprehensively exploited to access this variability as they contribute information on every region of the genome, regardless of the level of gene expression. Microsatellites (highly polymorphic simple sequence repeats) have been effectively exploited to enlighten bovine domestication and migration prototype (Bradley *et al*. 1994, Edwards *et al*. 2000) and to evaluate genetic diversity and relationships among cattle populations (MacHugh *et al*. 1997, Kim *et al.* 2002, Metta *et al.* 2004, Mukesh *et al.* 2004).

Purnea population of Indian zebu cattle (*Bos indicus*)

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evolved as a draught breed over centuries under low levels of breeding management. As a result of natural selection and human intervention Purnea animals adapted to harsh native environment, resistance to tropical diseases and external parasites and sustenance on low quality roughages and grasses. The breed is primarily employed for agricultural operations, for carrying load and transportation. The breeding tract of this breed encompasses Purnia commissionery of Bihar state. The breed is primarily maintained by small and marginal farmers. Bullocks are fast in speed and good for light draught (carting) and agricultural operations. The Purnea population in the breeding tract has been on a declining trend (Singh 2009). This calls for urgent conservation and improvement strategies for this breed. Thus the present study aimed to characterize Purnea cattle population at physical and molecular level.

MATERIALS AND METHODS

Survey of Purnea cattle in its breeding tract: The name of population has been derived from its place of origin i.e. Purnia district of Bihar. The Purnea cattle are mainly distributed Purnia, Araria, Katihar and Kishanganj districts.

Visits were made to different villages of its breeding tract to collect the information on Purnea cattle. The villages were selected in consultation with Bihar Veterinary College, Patna and State Animal Husbandry Department, Bihar. Information on the morphological traits (body length, height at wither, heart girth, paunch girth, horn length, ear length, face length and tail length without switch) were collected on 205 animals of different age and sex groups. Information on physical and performance traits and management practices was obtained through interview of cattle owners on a pre-designed questionnaire recommended for the purpose (http://www.nbagr.res.in/).

Sample collection and polymerase chain reaction: Blood samples were acquired from 50 random and unrelated Purnea animals from the breeding region following the guidelines of MoDAD (Measurement of Domestic Animal Diversity) programme (FAO 1995). To ensure unrelatedness of animals due to nonexistence of pedigreed account under field situations, animals were selected from distinct villages after interviewing the owners in detail. Blood samples (5–6 ml) were collected in vacutainer containing ethylene diamine tetra acetic acid (EDTA). Genomic DNA was extracted from whole blood using phenol-chloroform protocol (Sambrook *et al*. 1989). A panel of 23 bovine-specific microsatellite markers selected from the recommended list of the Food and Agricultural Organization (FAO 1998) and the International Society for Animal Genetics (ISAG) was used in this study for estimating within breed genetic variability in Purnea cattle.

Fig. 1. Geographical distribution of Purnea cattle in Bihar.

PCR was performed with 50–100 ng genomic DNA in a 25 µl reaction volume. 200µM of each dNTP, 50pMol of each primer and 0.5 units of Taq DNA polymerase was used for reaction. The reaction cycle was accomplished by denaturation for 1 min at 94°C; 30 cycles of 94°C for 1 min, precise annealing temperature of primer for 1 min, 72°C for 1 min and finally extension at 72°C for 5 min. The PCR products were then checked on 1.8% agarose gel followed by genotyping on ABI-3100 DNA sequencer. Genotypes were assigned using GeneMapper software package.

Data analysis: The data on phenotypic and morphometric traits were analysed as per standard statistical procedures described by Snedecor and Cochren (1989). The genetic data were analyzed using software GenAlEx 6.2 (Peakall and Smouse 2008) to calculate allele frequencies, observed (No) and expected number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population.

RESULTS AND DISCUSSION

This is the first attempt to generate information regarding the status of Purnea, a lesser known indigenous cattle populations of India in its breeding tract. An initial knowledge on the physical traits of the animals true to the population and distribution of genetic variability will be a great help in management, conservation and improvement programs of Purnea cattle.

Native environment: The breeding tract of Purnea cattle is located approximately 86°59'E to 88°19'E at longitude and 25°13'N to 27°07'N at latitude, covering approximately 12,000 sq.km. area. The elevation ranges from 20 m in Katihar to 47 m in Araria district. The forest cover in the breeding tract ranges from 5 sq.km. in Katihar district to 28 sq.km. in Araria district. The average annual rainfall ranges from 1,582 mm in Araria district to 2,250 mm in Kishanganj district. November to February are the cold months whereas, March to June are hot. Approximately 82% of the total rainfall occurs during monsoon season (July-Sep). In January, the minimum temperature goes down to 4°C whereas mean daily minimum temperature ranges from 5°C–10°C and mean

Fig. 2–5. **2–3.** Feeding practices for Purnea cattle in the native tract. 4–5. Type of houses for Purnea cattle in the breeding tract

maximum temperature ranges from 20°–25°C. During summer the maximum temperature raises to 48°C. Except in the first half of the summer, the humidity is above 70%. The Ganga, Kosi and Mahananda rivers flow in the breeding tract of Purnea cattle. Most of the area lies in the Gangetic plain therefore, the soil is mostly alluvial. However, light sandy soil is also seen in the area due to deposit of sand-silt by the rivers.

Major crops: Rice is an important crop in the area however jute, wheat, gram, barley, maize, masoor, arhar, khesari, peas, /sugarcane, tobacco, potato, chilies, moong, mustard, makkhana/ banana are also grown in that area.

Population status: The population status of the Purnea cattle is normal but showed a declining trend especially in last two decades. In the current census of livestock, 2007, the population of Purnea cattle has been reported to be 1,47,988 of which 139,488 are in Bihar and 8,500 in Jharkhand state.

Feeding: The animals are kept under zero or very low input system of management. The animals are sent for grazing during noon to evening hours. The bullocks and calves under 6 months of age were not sent for grazing.

The dry and green fodder was offered after chaffing with chaff cutter or locally made sharp knives. Little amount of concentrate (250 to 500g/d) was also offered to the animals by some farmers. The calves were fed from udder of their dams. The farmers used to feed the calves with the colostrum in the first fortnight of the calving.

Housing: The animals were kept in houses made nearby the farmers' houses during night. During day the animals were either sent for grazing or kept in open space. The feeding trough was made of cement and earthen material for providing them chaffed dry/ green fodder to them. The roof of animal houses was generally made up of thatch/ tin. The floor was *katcha* type. There was no drainage system provided in the animal houses. Half wall was found in most of the animal houses, which was generally made up of mud/ bamboo chips.

Breeding: In the remote village adequate numbers of Purnea breeding bulls were available for the breeding purposes through natural mating. However, in the area near by AI centers/ towns, the breed has been upgraded with the use of semen of exotic and indigenous milch cattle breed's bull (indigenous breeds: Hariana, Shaiwal, Red Sindhi, Tharparkar; crossbreds: Jersey and Holstein Friesian). The farmers were well aware of signs of estrous and right time of AI in the cows.

Castration of males: The castration of males is practiced at the age of 6 months to 2 years of age.

Herd size: The herd size varied from 3 to 25. Mostly the farmers were keeping about 5–10 animals.

Prophylactic measures and disease control: Purnea animals possess resistance to various infectious/ communicable diseases and ecto-parasitic infestations as compared to crossbreds. The vaccination cover to the animals was hardly 10–15% for hemorrhagic septicemia (HS) and 5% for foot-and-mouth disease (FMD). It was also informed that animals are comparatively more resistant to the FMD and if it happens, mouth lesions are less than foot lesions and animals are recovered within 3–4 days. HS is also less and there was less mortality among these animals as compared to the other groups of cattle and buffaloes.

Physical characteristics: The animals are of small size and compact body. The coat colour of Purnea cattle rangeds from light to deep red and some grayish white animals are also seen especially nearby district headquarters (Figs 6–10). Therefore, it would be more appropriate to call

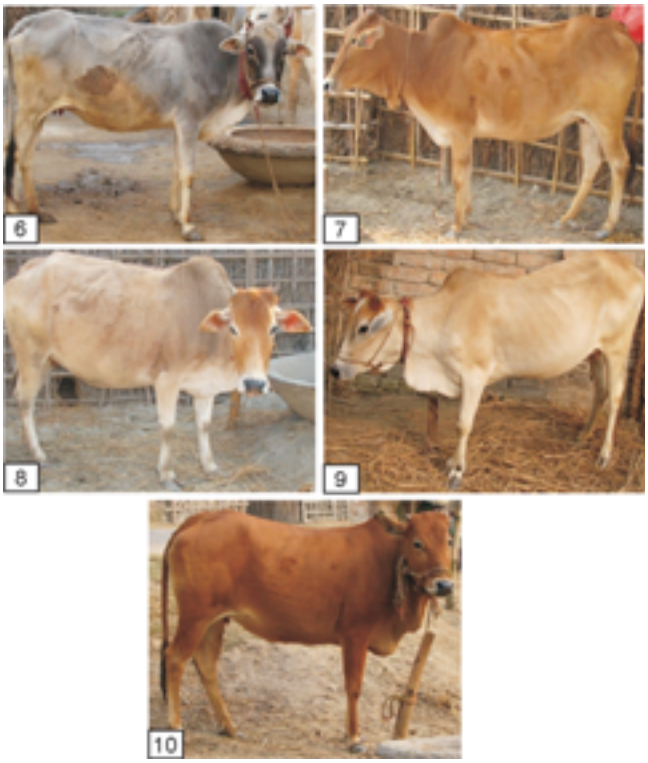

Fig. 6–10. Coat colour variation in Purnea cattle.

Table 1. Biometry (cm) of Purnea cattle

Trait	Cows	Bullocks	Young stock Male (1–3 years)	Young stock Female (1–3 years)	Calves (<1 year)
Body length	101.2±0.56 (84–114) N=88	104.2±1.61 (94–117) N=16	83.76±1.86 (69–102) N=21	86.97±1.03 (62–105) N=58	65.36±2.07 (50–82) N=22
Chest girth	131.6±0.99 (110–154) N=85	135.86±3.00 (115–156) N=14	103.05±2.62 (84–128) N=21	108.38±1.60 (78–134) N=58	76.91±3.03 (51–105) N=22
Height	103.31±0.58 (79–112) N=88	108.38±2.43 (82–128) N=16	90.05±1.64 (76–105) N=21	92.21±0.95 (71–110) N=58	73.27±2.25 (50–90) N=22
Paunch girth	136.4±1.26 (112–165) N=84	137.9±3.14 (122–158) N=14	104.67±2.76 (84–130) N=21	110.9±1.89 (73–142) N=58	75.32±3.24 (52–109) N=22
Face length	38.25±0.33 (18–47) N=87	41.31±0.66 (39–48) N=16	32±0.98 (21–41) N=21	33.12±0.43 (24–40) N=58	23.36±1.03 (16–30) N=22
Tail length without switch	69.97±0.75 (55–86) N=71	73.53±1.37 (64–84) N=15	53.37±2.0 (40–72) N=19	57.75±1.10 (38–79) N=56	38.90±1.86 (26–52) N=20
Tail length with switch	92.14±1.10 (67–113) N=71	98.13±1.94 (85–110) N=15	72.26±3.06 (50–96) N=19	76.55±1.34 (45–105) N=56	47.75±2.57 (30–65) N=20
Horn length	6.85±0.38 (1–18) N=86	8±1.32 (2–23) N=16	2.5±0.56 (1–5) N=10	2.63±0.25 (1–7) N=38	—
Ear length	20.01±0.18 (11–26) N=87	20.94±0.50 (18–25) N=16	18.14±0.30 (16–21) N=21	18.34±0.21 (13–22) N=58	14.95±0.55 (12–19) N=22

the breed as Purnea rather than local name of Red Purnea. The head profile was straight in almost all the females but slight budged fore head was seen in males. The face was triangular in appearance.

The colour of eyelid, muzzle and tail switch were mostly black. The body was short. The horns were also short, set in a crescent shape and point forward. The ears were horizontal in orientation. The limbs were short and thin. Majority of animals of Purnea cattle possessed small but well defined hump, which was better developed in males. The dewlap size was small to medium. The udders were small in size and bowl in shape. The teats were funnel shaped with pointed tips. The milk vein was poorly developed. The temperament of animals was docile.

Morphometric measurements: The body length, chest girth, height at withers, paunch girth, face length, tail length with and without switch, horn length and ear length were measured in 205 animals belonging to different age/ sex groups. The body measurements are presented in the Table 1. The body length, height at withers and chest girth of Purnea cattle is less then Bachaur cattle (Anonymous 2004) and Gangatiri cattle (Singh *et al*. 2007). This indicated that Purnea is the smallest breed of cattle found in Bihar. However, morphometric parameters of Purnea cattle are higher than the Malnad Gidda, dwarf cattle of Karnataka (Singh *et al*. 2008).

Performance: The age at sexual maturity in males was about 2.5 to 3.0 years and age at first calving of cows was found to be between 3 and 4 years of age. The average daily milk yield of cows was 0.5 to 2.0 kg. The lactation length varied between 5 and 8 months and calving interval ranged from 15 to 24 months. The bullocks of the Purnea were in good demand due to lower price.

Utility of the breed: The Purnea animals are playing a significant role in the rural livelihood of small and marginal farmers in terms of draught, milk and manure. The bullocks are well suited to agricultural operations in the area as bullocks of this breed can plough 0.3 to 0.6 acres of land in a day and can pull 4 q of load at the speed of 4 kmph. These are cheaper (Rs 5 to 10 thousand) as compared to Shahabadi or Bachaur bullocks (Rs 15 to 25 thousand). The dung of animals is used for fuel and manure purposes. Small amount of milk produced by the cows fulfill the requirement of family. Since these animals are well suited to the climate of the area and comparatively resistant to diseases and tolerant to the heat, the management of animals becomes cheaper to the farmers.

Genetic characterization: Across the 23 microsatellites scrutinized, a total of 204 distinct alleles were identified in Purnea. The allele frequency data revealed a reasonable amount of polymorphism in Purnea cattle (available from the authors on request). The number of observed alleles oscillated between 4 (ETH10, ILSTS11, BM1824) and 15 (TGLA53, MM12) with an overall mean number of alleles (MNA) per locus of 8.870±0.72. FAO has specified a minimum of 4 distinct alleles per locus for proficient judgment of genetic differences between breeds**.** All the 23 microsatellites employed in this study corresponded with this delineated principle and thus signified ample polymorphism and their suitability for evaluating genetic variation within breed and exploring genetic differences between breeds. The observed number of alleles for all the loci exceeded the

Table 2. Measures of genetic variation in Purnea cattle of Bihar

Locus	N	Na	Ne	Ho	He	F
ETH10	46	4	2.673	0.891	0.626	–0.424
ILSTS11	45	4	1.928	0.422	0.481	0.123
TGLA122	45	13	7.627	0.844	0.869	0.028
TGLA53	44	15	2.557	0.545	0.609	0.104
INRA05	43	6	4.251	0.581	0.765	0.240
INRA63	43	7	3.446	0.837	0.710	–0.179
MM08	43	8	2.703	0.605	0.630	0.040
TGLA227	43	10	1.871	0.302	0.466	0.351
CSSM08	31	7	2.409	0.581	0.585	0.007
HEL05	32	10	3.901	0.844	0.744	–0.135
ILSTS05	32	11	6.715	0.844	0.851	0.009
ILSTS33	32	6	2.864	0.531	0.651	0.184
INRA35	29	6	3.081	0.621	0.675	0.081
BM1824	47	4	2.297	0.404	0.565	0.284
CSSM66	47	14	5.084	0.745	0.803	0.073
ETH03	47	7	3.899	0.979	0.744	–0.316
ETH225	47	10	6.258	0.851	0.840	–0.013
MM12	47	15	4.909	0.787	0.796	0.011
CSSM33	23	13	7.007	0.826	0.857	0.036
HEL01	19	6	3.903	0.684	0.744	0.080
HEL09	24	10	8.000	0.833	0.875	0.048
ILSTS06	21	8	4.691	0.619	0.787	0.213
ILSTS34	23	10	4.054	0.652	0.753	0.134
Mean	37.087	8.870	4.179	0.688	0.714	0.043
SE	2.094	0.720	0.384	0.037	0.025	0.037

N, Sample size; No, observed no. of alleles; Ne, effective no. of alleles; Ho, observed heterozygosity; He, expected heterozygosity; F, F_{IS} = heterozygote deficiency.

effective number of alleles which varied from 1.871 (TGLA227) to 8.0 (HEL09) with a mean of 4.179±0.384 (Table 2). The mean observed number of alleles in this study for Purnea cattle population was similar in magnitude to Binjharpuri (8.8, Sharma *et al.* 2011). Higher mean number of alleles per locus was reported in some other indigenous-cattle Tharparker-9, Rathi-9.6 (Sodhi *et al.* 2008), Ghumsuri-9.4 (Sharma *et al*. 2011), Kumaun hill cattle-9.7 (Pandey *et al.* 2010) and Motu–10.04 (Pandey *et al.* 2011). However, lower mean number of alleles per locus was also reported in other Indian cattle breeds like Kherigarh-6.24 (Pandey *et al.* 2006a), and Bachaur-6.30 (Sharma *et al.* 2007).

The observed heterozygosity averaged over the 23 loci was 0.688±0.037 which was lower than the expected heterozygosity (Table 2). The average expected heterozygosity (Nei 1973) within the Purnea population diverged from 0.466 (TGLA227) to 0.875 (HEL09) with an overall mean of 0.714±0.025. Purnea cattle, thus, encompassed considerable measure of genetic variation derived from its gene diversity as estimated against the genetic variation described in several breeds scrutinized worldwide. Fairly comparable levels of heterozygosity were reported in 7 Italian cattle breeds 0.6 – 0.68 (Del Bo *et al.* 2001) and 5 Swiss cattle breeds 0.60–0.69 (Schmid *et al.* 1999). Heterozygosity of similar magnitude was also reported in Tharparker cattle of India (Ho = 0.64, He = 0.72) by Sodhi *et al.* (2008) and Orissa cattle populations (Ho = 0.62–0.66, He = 0.70–0.75) by Pandey *et al.* (2006) and Sharma *et al.* (2011). The average observed heterozygosity estimation in this study 0.688±0.037 is marginally higher than illustrated in Kenkatha (Ho = 0.540) by Pandey *et al.* (2006b), Bachaur (Ho = 0.534) by Sharma *et al.* (2007), Deoni (0.59) by Mukesh *et al*. (2004) and in exotic cattle: Brown Swiss (0.563) by Schmid *et al.* (1999) and 12 west/central African cattle breeds 0.506–0.697 (Ibeagha-Awemu *et al*. 2004). However, much lower heterozygosities (0.42 and 0.53) and reductions in number of alleles than Purnea have been recounted in 2 Indian zebu cattle breeds, viz. Sahiwal and Hariana (Mukesh *et al*. 2004) whose populations are on a rapid decline in India.

Within population inbreeding estimate f(F_{IS}) was significantly positive as derived from Table 1 wide randomizations (P< 0.05). The f-estimates ranged between –0.424 and 0.351 with an average of 0.043. Thus on an average a shortfall (4.3%) of heterozygotes existed in the Purnea population. All except 5 microsatellite markers, contributed to this observed heterozygote shortage. The F_{IS} values obtained in our study were lower than several other Indian cattle breeds and this was expected owing to more number of alleles and higher heterozygosity values. Higher heterozygote deficiency than the Purnea cattle (0.043) has been observed in several Indian cattle like Kumaun Hill cattle and Ghumsuri (F_{IS} = 0.08, Pandey *et al.* 2010, Sharma *et al.* 2011), Binjharpuri (F_{IS} =0.10, Sharma *et al.* 2011), Motu (F_{IS} = 0.113, Pandey *et al.* 2011), Sahiwal (F_{IS} = 0.32) by Mukesh *et al.* (2004), Bachaur (F_{IS} = 0.22) by Sharma *et al.* (2007) and Gangatiri (F_{IS} = 0.31) by Sharma *et al.* (2006).

Numerous factors, viz. inbreeding, locus under selection (genetic hitchhiking), null alleles (non amplifying alleles) and occurrence of population sub structure (Wahlund effect) were established as responsible for insufficiency of heterozygotes in a population (Nei 1987). Non-accessibility of adequate quantity of breeding bulls in the population could be the predictable basis for the ascertained deficiency of heterozygotes. Null alleles are largely unlikely to be segregating at all the loci. Likewise prospective Wahlund effects (localities with subpopulations) may not account significantly for the observed heterozygote deficit. The inbreeding detected in this population may be a manifestation of declining population size, coupled with lack of sufficient number of breeding males in the breeding region.

The significant level of variability in Purnea cattle is indicative of a valuable reservoir of genetic diversity in this population. This fact coupled with the evident environmental acclimatization emphasizes the instant inevitability for registration of Purnea population as a cattle breed, genetic improvement and its sustainable utilization. It is distinctively indispensable at this point of time to initiate planned and

organized breeding, as F_{IS} is indicative of heterozygote deficiency in the population. To make a start, breed society needs to be formed, which should be educated and supported for the comprehensive safeguarding and upgrading of the breed to make it economically sustainable in the moderating agricultural scenario of the country. Availability of proven males as well as frozen semen of the breed should also be ensured in the breeding tract.

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