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Characterization of Shahabadi cattle of Bihar— Phenotypic and molecular approaches

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In addition to 34 recognized breeds of cattle, various cattle populations exist in India, which have not yet been properly documented. Characterization of all such cattle populations is important to provide overall picture of cattle genetic diversity. Change in production system, mechanization, loss of rangeland grazing resources, inappropriate breeding policies, their implementation and indiscriminate introduction of exotic breeds are resulting in the disappearance of uncharacterized local populations of cattle, along with the unique gene and gene combinations. Shahabadi, Taylor, Bachaur and Red Purnea cattle are native to Bihar. Two decades earlier typical animals of these breeds were available in their native tracts in optimum number to constitute viable breeding populations. But at present, all of these populations need immediate attention, failing which our ancestral efforts of artificial selection, operative since time immemorial, may go in vein and there may be a non-repairable loss of environment as well as eco-friendly genes from the national and world gene pool (Singh 2009). For formulating any conservation plan, information about genetic diversity is a pre requisite. Thus, present study was undertaken to characterize Shahabadi cattle population for its phenotypic traits and assessment of genetic variation using microsatellite genotyping.

As per literature, the breeding tract of Shahabadi cattle is in the districts of Bihar, adjoining river Ganga. Visits were made to different villages of its breeding tract to collect the information on Shahabadi 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 175 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 for the purpose (http://www.nbagr.res.in/).

For estimating parameters of genetic diversity, fifty unrelated Shahabadi animals were randomly chosen from the breeding tract as per the guidelines of MoDAD (Measurement of Domestic Animal Diversity) programme (FAO 1995). Blood samples (5–6 ml) were collected in vacutainer containing ethylene diamine tetra acetic acid (EDTA) and genomic DNA was extracted 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 Geneticists (ISAG) for estimating within breed genetic variability in Shahabadi cattle. 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 consisted of 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.

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 effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population.

Breeding tract: The Shahabadi cattle are mainly

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Table 1. Morphometric parameters of different ages and sexes in Shahabadi cattle

Traits	Cows (N, 70)	Bullocks (N, 10)	Young stock (1–3) males (N,18)	Young stock (1–3) females (N, 45)	Calves (N, 32)
BL	123.80±0.39	127.10±0.94	97.44±2.34	106.36±1.77	82.81±1.84
CG	155.96±0.58	165.9±3.08	116.39±4.07	130.36±2.35	98.38±2.99
Height	122.4±0.58	129.8±1.66	103.33±2.23	108.76±1.25	91.13±1.82
PG	169.64±1.25	180.4±2.53	126.94±4.21	137.67±2.63	103.44±3.68
FL	44.91±0.20	47.7±0.80	37.39±1.16	40.71±0.66	31±1.12
FB	55.29±0.09	56.4±0.37	13.61±0.28	14.96±0.60	12.09±1.66
TWOS	83.56±0.99	92.2±2.46	64.61±2.20	69.51±1.72	54.53±1.66
TWS	106.1±1.49	113.9±4.17	79.44±3.14	86.98±2.10	63.47±2.14
HL	15.16±0.61	17.1±1.86	2.67±0.59	4.30±0.48	2.09±0.59
EL	27.39±0.17	28.3±0.52	24.5±0.64	24.96±0.30	21.75±0.48

BL, Body length; CG, chest girth; height, height at withers; PG, paunch girth; FL, face length; FB, face breadth; TWOS, tail length without switch; TWS, tail length with switch; HL, horn Length; EL, ear length.

distributed in Buxar, Bhojpur (Ara), Kaimoor (Bhabhua) and Rohtas (Sasaram) districts of Bihar. However, present survey revealed that the Shahabadi cattle are available in higher numbers in Bhojpur and Buxar districts as compared to other 2 districts (Fig.1). Since animals were mainly localized in Bhojpur, information on the morphological traits of animals were collected from different villages of Bhojpur district.

Fig. 1. Geographical distribution of Shahabadi cattle in Bihar

Morphological characteristics: Shahabadi cows have grayish white and white coat colours which the farmers refer as *sokan* and *dhawar*, respectively in local language. The bulls have white coat colour with greyish/blackish patches on hump and extremities. Muzzle, eyelids, switch of tail, hooves of animals is generally black. The horn colour is black or muddy. The orientation of horns is outwards, upwards, inwards and ending with pointed tips in majority of animals, whereas in some cases it is outward and upward with pointed tips and comparatively smaller in size. Ears are horizontal in orientation with mean length of 27.39 cm in cows and 28.3 cm in bullocks. The forehead is found to be straight. Hump and dewlap is medium, naval flap is small and basic temperament is moderate. Bowl shaped fore and rear udders, cylindrical teats with rounded tips are observed in Shahabadi cows with small to medium milk vein (Figs. 2–4).

Body measurements: Body measurements were recorded on 175 animals of different age and sex. All the data were analyzed according to age and sex. The average body length, height at wither, heart girth, paunch girth, horn length, ear length, face length and tail length without switch (Table 1). Singh *et al.* (2007) reported slightly lower body length, chest girth but higher height at withers for Gangatiri cattle as compared to Shahbadi cattle. Singh and Pundir (2007) also reported similar observations for Mewati cattle.

Figs 2–4. Shahabadi breeding bull, Shahabadi cows showing coat colour variation.

Fig. 5. Housing system in the breeding tract

Management and utility: Shahabadi animals are playing a significant role in the rural livelihood of small and marginal farmers in terms of draught, milk and manure. The draught ability of the bullocks of the breed was reported to be good with medium quantity of milk from cows. The veterinarian and livestock keepers informed that the animals are better in terms of disease resistance and reproductive performenace as compared to crossbred cattle of the area. The herd size varied from 2 to 30 animals. In villages farmers generally keep 2–3 cows and a pair of bullock. The animals are kept in open or *kachcha* (thatched) houses nearby the farmers' houses but *pucca* houses are available with some resourceful farmers. The floor of the animal houses is generally *kachcha* (Fig. 5). The animals are kept on grazing for 4 to 8 h in a day. The concentrate feeding/ balanced feeding is rarely practiced, however, paddy straw along with little amount (500g to 1 kg) of concentrate, prepared by using some cereals and groundnut cake at home, is given to the bullocks and also to cows at the time of milking.

Performance: The age at first service in bulls is 2.5–4 years, whereas, age at first calving in cows is reported to be 3.5–4.5 years. The milk production varies from 2 to 6 liters in a day but in rare cases it was observed to be 8 liters/day. The lactation period ranges from 6 to 10 months. The reproductive efficiency of animals is reported to be good as cows conceived in 1–2 natural mating. The production performance of animals is good upto at least first four lactations. The inter-calving period varies from 15 to 24 months.

Breeding: In villages under study, one to two bulls of the breed have been found. The bulls are not selected on the basis of their dam's performance or progeny testing. Farmers generally raise the healthy and good looking bull calves to develop them as bulls. Breeding, in majority of cases, was through natural mating. The farmers were well aware of signs of estrus and significance of artificial insemination. Some farmers also preferred the A.I of their cows. However it's important to mention that exotic/crossbred semen is available in the breeding tract whereas, Shahabadi bull semen is unavailable to the owners. This can be one of the causes for dilution of the Shahabadi population.

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

Locus	N	No	Ne	Ho	He	F_{IS}
ETH10	43	6	4.064	0.814	0.754	–0.080
ILSTS11	43	4	2.189	0.581	0.543	–0.070
TGLA122	43	13	8.722	0.791	0.885	0.107
TGLA53	43	15	3.539	0.628	0.717	0.125
INRA05	48	9	4.590	0.708	0.782	0.094
INRA63	48	6	2.706	0.563	0.630	0.108
MM08	48	8	3.080	0.625	0.675	0.075
TGLA227	48	14	1.840	0.417	0.456	0.087
CSSM08	44	6	3.396	0.545	0.706	0.227
HEL05	44	13	6.853	1.000	0.854	–0.171
ILSTS05	44	7	4.190	0.750	0.761	0.015
ILSTS33	44	8	4.008	0.705	0.751	0.061
INRA35	44	18	10.381	0.795	0.904	0.120
BM1824	48	8	2.733	0.500	0.634	0.211
CSSM66	48	12	5.097	0.854	0.804	–0.063
ETH03	48	14	6.857	0.979	0.854	–0.146
ETH225	48	8	1.875	0.521	0.467	–0.116
MM12	48	14	4.193	0.729	0.762	0.042
CSSM33	48	17	8.409	0.896	0.881	–0.017
HEL01	35	9	3.895	0.771	0.743	–0.038
HEL09	48	10	7.396	0.896	0.865	–0.036
ILSTS06	44	9	4.555	0.591	0.780	0.243
ILSTS34	48	13	4.712	0.729	0.788	0.074
Mean	45.522	10.478	4.751	0.713	0.739	0.037
SE	0.665	0.800	0.481	0.033	0.026	0.024

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

Genetic characterization: All the selected microsatellite loci (23) were polymorphic and a total of 241 alleles were observed (Table 2). Mean number of alleles observed over a range of loci is considered to be a reasonable indicator of genetic variation within the population (MacHugh *et al.* 1997). Sufficient allelic diversity was found in Shahabadi cattle with observed number of alleles varying from 4 (ILSTS11) to as high as 18 (INRA35) with overall mean number of alleles per locus as 10.48±0.8. The effective number of alleles ranged between 1.84 (TGLA227) to 10.381 (INRA35). The mean effective number of alleles (4.751±0.48) was lower than mean observed number of alleles (10.48±0.8). The observed number of alleles across all the loci was more than the effective number of alleles as per expectations. This is due to the low frequency of several alleles expressed at the locus. Thus the alleles having high frequency might have contributed to the overall allelic frequency. Nevertheless, the loci revealed high level of allelic diversity among them. The mean observed number of alleles in this study for Shahabadi cattle population was similar in magnitude to that reported in some other indigenous cattle

breeds/ populations, viz. Tharparker (9), Rathi (9.6, Sodhi *et al.* 2008) and Ghumsuri (9.4, Sharma *et al.* 2011), Kumaun hill cattle (9.7, Pandey *et al.* 2010), Motu (10.04, Pandey *et al.* 2011). However, lower mean number of alleles per locus was reported in other Indian cattle breed like Kherigarh (6.24) by Pandey *et al.* (2006a), Bachaur (6.30) by Sharma *et al.* (2007) and Binjharpuri (8.8) by Sharma *et al.* 2011.

A more appropriate measure of genetic variation within a population is gene diversity/ average expected heterozgosity (Nei 1987). The average observed heterozygosity was less than that of expected. The observed and expected heterozygosity values ranged from 0.417 (TGLA227) to 1 (HEL05) with the overall mean of 0.713±0.033 and 0.456 (TGLA227) to 0.904 (INRA35) with the overall mean 0.739±0.026, respectively. The higher heterozygosity pointed to allelic richness and higher gene diversity in the population. The quantum of genetic variation observed in the present study on Shahabadi cattle may be due to intermixing of different populations and/or to the low selection pressure. Heterozygosity of similar magnitude has also been reported in Tharparker cattle of India by Sodhi *et al.* (2008) and Odisha cattle populations by Pandey *et al.* (2011) and Sharma *et al.* (2011). Heterozygosity lower than Shahabadi cattle was been reported in Kenkatha (Ho = 0.540) by Pandey *et al.* (2006b), Bachaur (Ho = 0.534) by Sharma *et al.* (2007) and in exotic Brown Swiss cattle (0.563) by Schmid *et al.* (1999). For the markers to be useful for measuring genetic variation, average heterozygosity should range between 0.3 and 0.8 in the population (Takezaki and Nei 1996). The average expected heterozygosity value of 0.739±0.026 was within this range, which supports the suitability of battery of microsatellite markers used for the present study.

The estimate of heterozygote deficiency varied from –0.171 (HEL05) to 0.243 (ILSTS06) with a mean positive valves of 0.037. Thus it was observed that 3.7% deficiency of heterozygotes exists in Shahabadi cattle population. The heterozygote deficiency can be due to population sub division, genetic drift, selection against heterozygotes or inbreeding. The F_{IS} values obtained in our study were lower than other Indian cattle breeds and this was expected owing to more number of alleles and higher heterozygosity values (Table 2). Higher heterozygote deficiency than the Shahabadi cattle (0.037) has been observed in several Indian cattle like Kumaun Hill cattle and Ghumsuri (F_{IS} = 0.08, Pandey *et al.* 2010 and 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).

The genetic analysis based on selected microsatellite markers indicated sufficient genetic variability of Shahabadi population at present. Thus there is need of scientific management of the population so as to retain the founder alleles to the maximum possible extent. In view of the declining population of Shahabadi cattle in the breeding tract and shrinkage of the breeding tract itself, planning is to be done to increase the population hand in hand with maintaining the genetic purity.

SUMMARY

In the present study, Shahabadi cattle population of Bihar was characterized both phenotypically as well as at molecular level using microsatellite markers. A survey was conducted in different villages of Bhojpur and Buxar districts and morphometric data on 175 Shahabadi cattle was collected. Fifty unrelated Shahabadi animals were randomly chosen from the breeding tract for genotyping with 23 microsatellite markers on automatic DNA sequencer. A total of 241 alleles were observed with an average of 10.48±0.8 alleles per locus indicating reasonable amount of polymorphism in Shahabadi cattle. The average observed heterozygosity (Ho) varied from 0.417 (TGLA227) to 1 (HEL05) with a mean of 0.713±0.033 which was less than the expected heterozygosity (He). An average of 3.7% heterozygote deficit was detected in the Shahabadi population. Keeping in view the low input required by animals and contribution in the rural livelihood, there is a need to take up systematic genetic improvement programme of the breed so as to make it more sustainable in present management system.

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