

Morphometric Characterization and Molecular Identification of Different Cattle in Some Selected Regions of Bangladesh

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ABSTRACT: In order to determine the morphometric characterization and molecular identification of cattle in Bangladesh, three districts, i.e., Pabna, Bogra and Jhenidah were selected. In each district, 15 outstanding different crossbred and local dairy female cattle's (cows) blood samples (total 45) and raw data were collected to carry out the study of molecular and morphometric characters respectively. These characters were observed to identify the genetic resources of cattle in the selected regions such as eye color, coat color, horn pattern, age, breed types, conception rate, litter size, milk production, lactation length, heat period and gestation period. The quantity of DNA were found to 198.30 ± 102.40 $\mu\text{g/ml}$, 193.70 ± 86.34 $\mu\text{g/ml}$ and 196.88 ± 91.54 $\mu\text{g/ml}$ in Pabna cattle, Jhenidah cattle and Bogra cattle respectively. The purity of DNA was also analyzed and observed 1.80 ± 0.25 , 1.84 ± 0.15 and 1.64 ± 0.22 (OD) in Pabna cattle, Bogra cattle and Jhenidah cattle accordingly. For RAPD experiment, nine primers were randomly tested to evaluate their suitability for amplification of the DNA sequences among these two were matched and found polymorphic, BMC-1222(CCTGAGTGTTCTCCTGAGT) and OPB-07(GGTGACGACG). By studying the different molecular and morphometric data it was found that the Pabna cattle possess better genetic resource rather than two other districts like Bogra and Jhenidah. The RAPD profile of PCR amplification in this study can be a useful tool for detecting molecular diversity and genetic variation. Thus, these data give an accession or set of accessions and also helpful for germplasm conservation and improvement program of cattle in our country.

KEYWORDS: Cattle, crossbred, morphometric, livestock, marker.

1 INTRODUCTION

The livestock sector in Bangladesh is one of the major components of agricultural output (crops, livestock, fisheries and forestry) which plays a significant role in national economy, gross domestic product (GDP) and foreign exchange earnings [1]. Bangladesh possesses about 24.5 million cattle, which is about 1.79% of the world and 5.47% of Asian cattle population [2]. The total ruminant livestock population of Bangladesh is composed of 24.0 million cattle among them 34.4 million goats, 0.83 million buffalos and 1.14 million sheep [3]. The local as well as improved varieties of cattle breeds are also available in Bangladesh. The classification of the indigenous cattle varieties is primarily based on some morphological and physiological attributes such as body size, horn shape and size, coat color and hump size and their geographical distribution. But there is a little documentation of these varieties on behalf of its diverse resources. However, the usefulness of phenotypic traits to study the genetic variation between populations is very limited [4]. Cattle genetic resources are one of the main tools for cattle variety development. The qualitative and quantitative improvement of these cattle mainly relies on the available gene pool and its manipulation. A number of DNA based marker system are now available for genetic evaluation, classification, characterization and data documentation. Molecular markers based on DNA sequence are more reliable in this regard such as RAPD, RFLP, and AFLP etc. The RAPD marker, generated by the polymerase chain reaction (PCR) is widely used since 1990's to assess intra-specific genetic variation at nuclear level [5], [6]. Therefore, molecular markers can aid estimating the genetic variability of species, breeds and populations, as well as decision related to selection of breeds/ populations to be conserved

[7]. In the absence of proper genetic identification, the risk of loss of surviving genetic diversity is high. Unlike morphological traits, molecular markers which are assumed to be neutral to the selection forces are appropriate in the study of genetic relationship between breeds [8]. Classification using molecular markers provides a large unbiased basis for the estimates of average breed similarities and differences. Thus, the techniques for the analysis of the genetic variability are an essential ingredient for the programs of rational conservation and improvement, since they must be based on the combination of the phenotypic and genetic data [9]. Therefore, the current research work has been undertaken to determine the morphometric traits, molecular identification & variation and the genetic status of cattle in the selected regions of Bangladesh that would be helpful for their future improvement and conservation program.

2 MATERIAL AND METHODS

The research was conducted in the Biotechnology Laboratory under Animal Production & Research Division of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh. This experiment has two parts: A) Morphometric Study & B) Molecular study.

2.1 LOCATION OF THE STUDY AREA

Three regions namely Pabna, Bogra and Jhenidah were selected for morphometric and molecular studies. A total number of 45 outstanding different crossbred and local dairy female cattle blood samples were collected in order to carry out the experiment, beside this phenotypic traits of those cattle also noted. Out of the 45 cattle blood samples 15 from Pabna, 15 from Bogra and 15 from Jhenidah were considered.

2.2 COLLECTION OF DATA (MORPHOMETRIC STUDY)

The information's of productive and reproductive performances of each cow was collected by face to face interviewing the farmers by frequent visit and examination of the cow. For evaluation, following traits of cattle were considered: eye color, coat color, horn pattern, age, breed types, conception rate, heat period, litter size, milk production, lactation length and gestation period. The conception rates (CR) are calculated by dividing the number of cows confirmed pregnant (PR) from those breeding by the total number of cows inseminated [10].

2.3 COLLECTION OF BLOOD SAMPLES (MOLECULAR STUDY)

A total of 45 blood samples were collected from three different selected regions. At least 10ml of fresh blood sample was collected from each of the animal aseptically by puncturing jugular vein in EDTA containing Vacutainer and kept on ice. Subsequently the blood samples were transported to the laboratory and stored at -20°C until the study start.

DNA was isolated with small modification of the protocol of Roe et al. 1996 [11]. Briefly, 500ul blood samples were added with Standard Saline Citrate (SSC) Buffer (400 μl , 800 μl and 1000 μl) and centrifuged (13,000 rpm for 5 minutes) three times and supernatant was discarded every time. After that added 375 μl of 0.2 M Sodium acetate solution and vortex thoroughly. Then 25 μl of 10% SDS and 5 μl Proteinase-K were mixed and incubated at 55°C for 1 hour. Then 20 μl PCIA solution was added and mixed and centrifuged at 13,000 rpm for 5 minutes. After removing aqueous layer to new micro centrifuge tube, 1000 μl of 100% ethanol was mixed and then centrifuged at 13,000 rpm for 4 minutes and supernatant was discarded. Then 180 μl of TE buffer (10:1) was mixed and incubated at 55°C for 10 minutes. After mixing 20 μl of 2 M sodium acetate and centrifuged at 13,000 rpm for 4 minutes, the supernatant was discarded. Then 1000 μl of 70% ethanol was added and centrifuged again at 13,000 rpm for 4 minutes. After discarding the supernatant the pellets were dried in DNA concentrator by 15 minutes and then 150 μl TE buffer (10:1) was added. After proper mixing with TE buffer samples were incubated overnight at 55°C . At the end of incubation, samples were vortex properly and Store at -20°C for further study.

The isolated DNA sample was evaluated both qualitatively and quantitatively. Confirmation of isolated DNA can be done by comparing sample DNA with the standard DNA on 1% agarose gel electrophoresis staining with ethidium bromide or by estimating the absorbance of DNA by spectrophotometer at 260 nm. Both the methods were carried out in this study. Quantification by UV-spectrophotometer, 2ml of deionized sterile water and 2 μl of extracted DNA samples were taken in a cuvette and mixed properly. Then cuvette was settled at spectrophotometer and observations were taken at 260nm and 280nm absorption angle. Double stranded DNA is calculated by using the following formula [12]:

$$\text{Concentration of DNA} = \text{Absorbance} \times \frac{\text{Volume of distilled water } (\mu\text{l})}{\text{Amount of DNA } (\mu\text{l})} \times \text{Conversion factor } (0.05) \times 1000$$

2.4 PRIMER SELECTION

Nine primers were randomly tested to evaluate their suitability for amplification of the DNA sequences; of them two were matched and found polymorphic primers were evaluated based on intensity of bands, consistency within individual, presence of smearing, and potential for population discrimination.

Table 1. Randomly matched primer

Primer Code	Primer sequence 5'-3'	GC content %
B MC- 1222	CCTGAGTGTTCCCTCTGT	55
OPB - 07	GGTGACGCAG	70

2.5 PCR AMPLIFICATION BY RAPD MARKERS

The amplification conditions were based on Williams et al. (1990) [6], with some modifications. PCR reactions were performed on each DNA sample in a 25 μ l reaction mix containing 2.5 μ l of 10X Ampli PCR buffer, 1 μ l of 100 μ M primer, 4.0 μ l of 2.5mM dNTPs, 5 unit (0.2 μ l) of Ampli Taq DNA polymerase, 1.25 μ l of 50mM MgCl₂ and 4 μ l template DNA and suitable amount of sterile de-ionized water. PCR thermo cycler conditions were: 3 minutes preheated at 94°C, followed by 30 cycles of 1minute denaturation at 94°C, 1 minute annealing at 38°C and elongation or extension of the primer at 72°C for 2 minutes. After the last cycle, a final step of 10 minutes incubation at 72°C was done. After completion of cycling program, reactions were held at 4°C.

2.6 AGAROSE GEL ELECTROPHORESIS

PCR products from each sample were separated by running 2% agarose gel containing 12 μ l ethidium bromide in 1X TAE buffer at 80V, 300mA and 300W for 2 hrs. 1x Gel loading dye (3 μ l) was added to the PCR products and loaded in the wells. A molecular weight markers DNA (100 bp ladder DNA) were also loaded on either side of the gel. DNA bands were observed under UV light on a UV trans-illuminator and photographed by a camera.

2.7 DATA OF RAPD

RAPD were scored according to the presence or absence of polymorphic DNA fragments. The scores obtained using the primers in the RAPD analysis were then combined to create a single data matrix. The proper data were then taken for analyzing the similarity & variation determination.

2.8 STATISTICAL ANALYSIS

Results were expressed as mean \pm SD (Standard deviation of mean). ANOVA single factor was followed by Duncan's multiple ranges for multiple comparison tests. The statistical program used was Microsoft Office Excel 2007 and its add-in DSAASTAT (Andera Onofri, Dipartimento di scienze Agrarie Ambientali, Borgo xx Giugno, 7406121 Perugia, Italy). P < 0.05 was considered statistically significant.

3 RESULTS

Morphometric traits of 45 female cattle (15 from Pabna, 15 from Jhenidah and 15 from Bogra) were studied basically on body appearance, breed types, coat color, eye color, horn pattern and hump content. It was observed that all the selected cattle in Bogra and Pabna districts were found crossbred. On the other hand, in the Jhenidah district half of selected cattle were belongs to crossbred and the rest half were local type (Figure 1). Breed types varies on geographic area, different environmental factors and available source of natural feed.



Fig. 1. Different types of cattle: Crossbred cattle of Pabna & Bogra and Local cattle of Jhenidah

Table 2. Analysis of some morphometric characteristics

Region	Coat color variation of cattle					Breed types	
	Black	Red	Black- red	Black-white	White	Cross	Local
Bogra cattle	7	-	-	8	-	15	-
Pabna cattle	4	2	3	6	-	15	-
Jhenidah cattle	6	3	-	2	4	7	8

Table 3. Analysis of some morphometric characteristics

Region	Eye color		Horn pattern			Hump	
	Black	Ash	Small	Medium	Large	No	Yes
Bogra cattle	15	-	6	6	3	15	-
Pabna cattle	15	-	3	8	4	15	-
Jhenidah cattle	14	1	8	4	3	13	2

Table 4. Analysis of some productive and some reproductive characters

Region	Milk production (liters/day)	Lactation length (day)	Conception rate%	Litter size	Heat period (days)	Gestation period (days)
Bogra cattle	8.7±0.98 ^b	290±9.43 ^a	75±0.14 ^a	1.3±0.38 ^b	22.8±0.9 ^a	285.5±0.4 ^a
Pabna	14.6±1.34 ^a	284±8.50 ^a	80±0.41 ^a	3.3±0.84 ^a	23.5±0.5 ^a	285.5±0.4 ^a
Jhenidah	3.1±0.58 ^c	275±6.41 ^b	70±0.52 ^b	2.5±0.68 ^{ab}	18.9±0.7 ^b	280.3±0.2 ^b

Result are expressed as mean ± SD (Standard deviation), n= 15 sample of each region. Values in the same row with different superscripts (a,b,c) are significantly different at p< 0.05. ANOVA single factor followed by Duncan's multiple ranges for multiple comparison tests.

Table 5. Determination of DNA concentration and DNA purity of selected three regions

Region	DNA concentration (µg/ml)	DNA purity (A ₂₆₀ /A ₂₈₀)
Bogra cattle	196.88± 91.54 ^a	1.80±0.25 ^b
Pabna cattle	198.30±120.40 ^a	1.84±0.15 ^{ab}
Jhenidah Cattle	193.70±86.34 ^b	1.64±0.22 ^b

Result are expressed as mean \pm SD (Standard deviation), n= 15 sample of each region. Values in the same row with different superscripts (a,b,c) are significantly different at $p < 0.05$. ANOVA single factor followed by Duncan's multiple ranges for multiple comparison tests.

RAPD PRIMER SCREENING

Nine primers were randomly screened for DNA polymorphism. Out of them two primers OPB-07 and BMC-1222 were responded to polymorphic DNA. With the analysis of OPB-07 primer, sizes of different polymorphic DNA are obtained by comparing with the marker DNA at different stage.

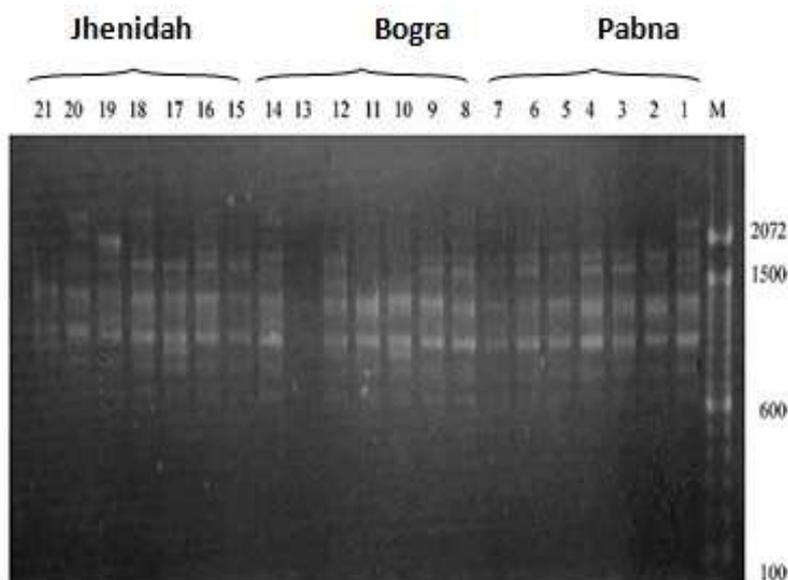


Fig. 2. RAPD banding pattern (primer OPB-07) of the cattle DNA sample ; 1-7 for Pabna, 8-14 for Bogra and 15-21 for Jhenidah

4 DISCUSSION

Local cattle are generally making cross to a foreign cattle (New Zealand or Australian cattle of good heredity) and produce a native crossbred cattle. Therefore, its productivity (eg. milk, meat etc) is generally high then local cattle and they are also susceptible to disease resistant and environmental issue. Coat color of selected cattle in Bogra region was mainly black and black-white in color. Pabna crossbred cattle were also black and black- white in color; but red and black cattle were also observed. On the other hand, selected local and crossbred cattle in Jhenidah were found black, red, black-white and white in color (Table 2). All of the cattle eye color was found black except only one cattle was found ash color in Jhenidah region. Horn pattern of cattle were found small, medium and large in size both of the selected region. The cows are generally not containing hump but only two cows were found to contain medium size of hump within the local breed types in Jhenidah region. The coat color of the variety is red or grey or a mixture of both (table 2). Al-Amin et al. (2007) [13], studied on North Bengal Grey (NBG) cattle of Bogra district and observed that the coat color of these animals is deep grey to white. The observed coat color was similar to the present study.

The Pabna cows are good in size and possess better milking ability [13], the average milk production of Pabna cattle was found 14.6 ± 5.34 lit/day that is better than the other two regions; Bogra for 8.7 ± 2.58 and Jhenidah for 3.1 ± 1.58 lit/day respectively (Table 3). Similar result was also observed by Udo et al. (1990) [14], who reported that Pabna crossbred cattle gives 30% more milk than Pabna local cattle. Some small to big dairy milk factories are established around Pabna district. The average lactation lengths of the selected cattle were 290 ± 9.43 , 284 ± 13.50 and 208 ± 43.41 days for Bogra, Pabna and Jhenidah areas respectively (table 4). Auld et al. (2007) [15], analyzed the effects of varying lactation length on milk production capacity of cows and found the intervals of 12 to 24 month. Al-Amin et al. (2007) [13], found the lactation length of 219 days for Bogra local cattle that did not agreed with crossbred cattle. However, Bag et al. (2010) [16], observed that the

maximum and minimum lactation length of RCC cattle was 295 days and 137 days respectively, that result is more or less similar to the present studied areas.

Conception rate of Bogra, Pabna and Jehnidah cattle region were analyzed, where Jehnidah cattle were found higher conception rate (1.9 ± 1.10) comparatively than Bogra cattle (1.2 ± 0.42) and Pabna cattle (1.2 ± 0.41) (table 4). Inadequate proper nutrition prior to calving, results in cows being thin at calving which delays the onset of estrual activity post-calving. This delay in onset of cycling activity will affect the percent of cows available to be bred during the breeding season, thus reducing overall conception rates. Conception rates of lactating cows decreased sharply when maximum air temperature on day after insemination exceeded 30°C [17].

The litter size is the number of offspring produced at one birth by a viviparous animal. Viviparous animal are those whose embryo development occurred inside the body of the mother, eventually leading to live birth, as opposed to laying eggs. The litter size of cattle found 1.3 ± 0.38 , 3.3 ± 0.84 and 2.5 ± 0.68 Bogra cattle, Pabna cattle and Jehnidah cattle respectively. Heat period and gestation period recorded higher in Bogra and Pabna district cattle. These heat period figures were 22.8 ± 0.9 and 23.5 ± 0.5 in Bogra and Pabna district cattle accordingly, and the gestation period were the same of these two districts, these figures were 285.5 ± 0.4 . In 2003, Talukder [18], revealed that the gestation period was 279.58 ± 1.85 days in BAU dairy farm. Another research work conducted by Khan et al. (2000) [19], and they observed the period of gestation in rural conditions was 282 days and in farm conditions was 281 days. Azizunnesa also [20], found the mean gestation length was 273.08 ± 7.48 days in farm condition in crossbred cows. The standard gestation period of cows is 285 ± 5 days [21].

For molecular study DNA was extracted from the blood samples by small modification of the protocol of Roe et al. 1996 [11]. The average concentration of DNA samples ($\mu\text{g/ml}$) of three variable regions of cattle were 198.30 ± 102.40 for Pabna, 193.70 ± 86.34 for Jehnidah and 196.88 ± 91.54 for Bogra, (table 5). The average quantity of DNA for Pabna region was higher than that of others two regions of DNA sample.

The average purity of extracted DNA in Pabna Cattle, Bogra Cattle and Jehnidah Cattle were 1.84 ± 0.15 , 1.80 ± 0.25 and 1.64 ± 0.22 respectively (Table 5). For pure double-stranded DNA, Sambrook et al. 1989 [22], established that the ratio A260/A280 should be within 1.8 to 1.9.

5 CONCLUSION

In conclusion that can be drawn from this research work is that the morphometric data obtained in this study might be useful tool in future strategies for cattle breeding. RAPD data would also be useful for detecting genetic diversity & inter-relationship among them, which would be utilized with right protection purposes and also helpful for germplasm conservation from the best genetic resource areas. The present study also finds the molecular technique for quantifying genomic DNA and its purity among the cattle varieties, although it needs for a small work with a greater number of genotypes using greater number of primers for better understanding of the genetic variability of the cattle varieties in the country.

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