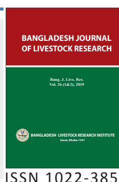
An Official Journal of Bangladesh Livestock Research Institute (BLRI)

Bangladesh Journal of Livestock Research

Journal Homepage: https://www.banglajol.info/index.php/BJLR



ISSN 1022-3851

Genetic variations among individuals of BLRI Cattle Breed-1 in successive generations

M.P. Mostari*, M.Y.A. Khan and G.K. Deb

Animal Production Research Division
Bangladesh Livestock Research Institute, Savar, Dhaka.

Abstract

This study aimed to identify the genetic variation among different generations of BLRI Cattle Breed-1 (BCB-1) and its relationship with indigenous Pabna cattle. A total of 20 ISAG-FAO recommended microsatellite DNA markers were used for this study. Blood samples were collected randomly from fifteen animals of 1st, 2nd, 3rd and 4th generation of BCB-1, Pabna cattle and RCC as reference cattle breed. The DNA extraction, polymerase chain reaction (PCR) amplification by using microsatellite markers and finally capillary based fragment length analysis was performed by genetic analyzer. The allele number per locus ranged from 5 to 14 in the studied populations. The mean numbers of alleles ranged from 4.95 in 3rd generation of BCB-1 to 7.55 in indigenous Pabna cattle. The mean numbers of alleles per locus decreased in 3rd and 4th generation. Both observed and expected heterozygosity decreased in subsequent generations in BCB-1. The observed heterozygosity decreased in BCB-1 from 2nd to successive generations (0.7257 in 2nd generation to 0.6232 4th generation). The average observed heterozygosity was highest in indigenous Pabna cattle (0.7393). Genetic distance between 3rd and 4th generation of BCB-1 are very low they are more homogenous. Indigenous Pabna cattle and RCC showed more genetic distance from all generations of BCB-1. Dendrogram based on DA genetic distance using unweighted pair group method of arithmetic means (UPGMA) indicated segregation of the four generations of BCB-1 into cluster 1 and 2 which are grouped together and separated from the cluster 3 of indigenous Pabna cattle and cluster 4 RCC population. According to genetic structure of BCB-1, it may treat as a unique cattle population of Bangladesh.

(**Keywords:** BCB-1, heterozygosity, microsatellite marker, genetic distance)

Bang. J. Livs. Res. Vol. 26 (1&2), 2019: P. 8-14 https://doi.org/10.3329/bjlr.v26i1-2.49932

Introduction

Bangladesh Livestock Research Institute (BLRI) has developed BLRI Cattle Breed-1 (BCB-1) through selective breeding within indigenous cattle of greater Pabna district, which was evolved through admixture of Hariana, Tharparker and Sahiwal genetic materials with local cattle (Bhuiyan, 2008). BLRI collected these cattle and have been conserving them Ex *situ* since 1992. The selective breeding program has been conducting to improve milk and meat

*Corresponding author: adiba_blri@yahoo.com

production performance of the genotype over the last 28 years. Selection pressure was put on lactation yield, live weight and red coat color of animals. These selection pressures resulted changes in the physical appearance and production performance of the cattle toward a distinct feature than that of their ancestors (Deb *et al.*, 2009). The live weight of mature BCB-1 bulls ranged from 450 to 550 kg and cows ranged from 250 to 350 kg at the age varies from 5 to 6 years. The cows produce 900-1000 liters of milk in a lactation period of 240-260 days (Deb *et al.*, 2008 and 2009). The coat of the breed is red in color with a gradual shift to fawn or light brick at the ventral part of the body. The progress in milk production potential was very low. However, the attractive coat color and body size of the bull has a great market value. Over the last couple of years, BLRI systematically studied their morphological, production, reproduction, nutritional management, disease prevalence and beef characteristics (Deb *et al.*, 2008 and 2009). At present situation, the BCB-1 herd of BLRI research farm is progressed to 4th generation and few calves of 5th generation have already borne. However, the genetic relationship of BCB-1 among its different generations and with its progenitor Pabna Cattle is still unknown. Considering these facts, this study is aimed to identify the genetic variation among four generations of BCB-1 and its relationship with Pabna cattle using microsatellite DNA marker where, Red Chittagong Cattle (RCC) was used as reference cattle breed because RCC has already characterized in different studies (Bhuiyan *et al.*, 2005, Bhuiyan *et al.*, 2007, Habib *et al.*, 2009, Habib *et al.*, 2012, Hossain *et al.*, 2018)

Material and Methods

Sample collection, DNA extraction and quantification

Blood samples were collected from 1st to 4th generation of BCB-1 from the BLRI cattle research farm and Pabna Cattle form the

Table 1. Number of samples collected from different cattle populations in different areas

Cattle Populations	Number of collected samples	Area s of sample collection
BCB-1 1st generation	15	BLRI cattle research farm, Savar, Dhaka.
BCB-1 2nd generation	15	BLRI cattle research farm, Savar, Dhaka.
BCB-1 3rd generation	15	BLRI cattle research farm, Savar, Dhaka.
BCB-1 4th generation	15	BLRI cattle research farm, Savar, Dhaka.
Pabna cattle	15	Greater Pabna District (Pabna and Sirajgonj)
Red Chittagong cattle (RCC)	15	BLRI cattle research farm, Savar, Dhaka.

greater Pabna District. The reference RCC sample were collected also from the BLRI cattle research farm. Fifteen blood samples including males and females were collected from unrelated animals of each generation of BCB-1, Pabna cattle and RCC. Blood samples were collected in EDTA containing 4 ml vacutainer using 21G needle. The vials were gently tilted for mixing the blood with anticoagulant. The vial was marked with the date of collection, sex and genotype. The blood samples were carried into lab in ice box and stored at -20°C until extraction of genomic DNA (gDNA). The gDNA was isolated from blood using a commercial kit (QIAGEN DNA Mini Kit) following manufacturer instruction. The gDNA was quantified using 1% agarose gel electrophoresis and Nanodrop 2000 spectrophotometer.

International Society of Animal Genetics and Food and Agriculture Organization (ISAG-FAO) recommended 20 microsatellite DNA markers (Table 2) were used for the PCR amplification and genetic variation study. The PCR amplification was done at Molecular Genetics Laboratory of Animal Production Research Division, BLRI and microsatellite genotyping (Fragment Length Analysis) was performed in a commercial laboratory named as First Base, Malaysia (www.base-asia.com). All gDNA samples were amplified in PCR machine using 20 pairs of selected microsatellite primers. All the microsatellite markers were forwardly labeled with a capillary based dye: FAM (blue), HEX (Green), TAMARA (yellow) and ROX (Red) for the purpose of genotyping. PCR was carried out in 15µl reaction mixture containing 4ng/µl of template DNA, 0.3µl each 10µM primer, 1µl of 20 mM dNTPs, 0.05 unit/µl of *Taq* DNA polymerase, 1.2 to 1.5mM/µl of MgCl2 and 1

Table 2. Selected ISAG-FAO recommended microsatellite markers

SL. No.	Name	Primer sequence (5′-3′) Forward Reverse	Gene bank accession number	Allele range (bp)
1	INRA063	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	X71507	167-189
2	CSSM66	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	...	171-209
3	INRA037	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	X71551	112-148
4	INRA005	CAATCTGCATGAAGTATAAATAT CTTCAGGCATACCCTACACC	X63793	135-149
5	HAUT27	AACTGCTGAAATCTCCATCTTA TTTTATGTTCATTTTTTGACTGG	X89252	120-158
6	TGLA227	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	...	75-105
7	BM1824	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	G18394	176-197

SL. No.	Name	Primer sequence (5′-3′) Forward Reverse	Genebank accession number	Allele range (bp)
8	ETH152	TACTCGTAGGGCAGGCTGCCTG GAGACCTCAGGGTTGGTGATCAG	G18414 Z14040	181-211
9	HEL9	CCCATTCAGTCTTCAGAGGT CACATCCATGTTCTCACCAC	X65214	141-173
10	BM1818	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	G18391	248-278
11	ILSTS006	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	L23482	277-309
12	ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	L23481	176-194
13	INRA035	TTGTGCTTTATGACACTATCCG ATCCTTTGCAGCCTCCACATTG	X68049	100-124
14	MM12	CAAGACAGGTGTTTCAATCT ATCGACTCTGGGGATGATGT	Z30343	101-145
15	INRA032	AAACTGTATTCTCTAATAGCTAC GCAAGACATATCTCCATTCCTTT	X67823	160-204
16	HAUT24	CTCTCTGCCTTTGTCCCTGT AATACACTTTAGGAGAAAAATA	X89250	104-158
17	BM2113	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	M97162	122-156
18	ETH225	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	Z14043	131-159
19	TGLA122	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	...	136-184
20	ETH10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	Z22739	207-231

X PCR buffer. All PCR products were quantified by 2% agarose gel electrophoresis for checking the success of PCR amplification. The amplified DNA was genotyped by automated capillary DNA sequencer (Genetic Analyzer 3730 of Applied Biosystem). The internal standard was prepared by adding 7.55µl of LIZ size standard to 0.45 µl of HiDi formamide. A total of 2µl of diluted PCR product of each co-loading was transferred to individual wells with 8µl of standard/formamide mix and denatured for genotyping. The genotyping results were processed by the Gene Mapper v4.0 program which determines the allele sizes in each animal. A total of 90 samples, 15 samples from each population were amplified and genotyped.

Data analysis

Allele frequencies at each locus for each

population, mean number of alleles per population and heterozygosity values (expected and observed) were calculated using genotyped data in Microsatellite Toolkit program (Minch, 1995). Dispan program (Ota, 1993) was used to calculate DA genetic distances between the cattle populations. An unweighted pair group-method with arithmetic mean (UPGMA) phylogenetic tree was constructed using the PHYLIP 3.57c package (Felsenstein, 1995).

Results and Discussion

The four generations of BCB-1, Pabna cattle and RCC showed polymorphism for microsatellite markers in the study. The allele number per locus ranged from 5 to 14 in the studied populations. The mean numbers of alleles (MNA) ranged from 4.95 to 7.55. The MNA per locus decreased in 3rd and 4th generation compared to 1st and 2nd generation of BCB-1. The reference cattle population RCC showed 5.70 MNA which is lower than the previous studied 6.22, by Bhuiyan *et al*. (2005). Both observed and expected heterozygosity decreased in subsequent generations among BCB-1. The observed heterozygosity of BCB1-G2 was 0.7257, which reduced to 0.6232 in BCB1-G4. The average observed heterozygosity values ranged from 0.6232 to 0.7393 with the lowest in BCB1-G4 (0.6232) and the highest in Pabna cattle (0.7393). Observed heterozygosity in RCC was 0.6829. The mean numbers of alleles along with observed and expected heterozygosity values per cattle population are presented in Table 3.

In the table 4 the genetic distance among the four generations of BCB-1 is comparatively lower than the genetic distance between the four generation of BCB-1 and its progenitor Pabna Cattle. RCC has higher genetic

Table 3. Cattle population statistics

Cattle Populations	Genotyped sample no.	Loci typed	Mean number of Alleles (MNA)	Observed Heterozygosity	Expected Heterozygosity
BCB1-G1	15	20	5.45±2.04	0.6731±0.0291	0.6918±0.0359
BCB1-G2	15	20	5.75±1.55	0.7257±0.0258	0.7049±0.0291
BCB1-G3	15	20	4.95±1.23	0.6750±0.0280	0.6508±0.0378
BCB1-G4	15	20	5.30±1.95	0.6232±0.0291	0.6462±0.0484
Pabna cattle	15	20	7.55±1.93	0.7393±0.0254	0.7828±0.0209
RCC	15	20	5.70±1.78	0.6829±0.0269	0.7149±0.0290

Table 4. Genetic distance between cattle population

	BCB1-G1	BCB1-G2	BCB1-G3	BCB1-G4	Pabna Cattle
BCB1-G2	0.0867	0			
BCB1-G3	0.0949	0.0877	0		
BCB1-G4	0.0966	0.1010	0.0810	0	
Pabna Cattle	0.1788	0.1574	0.1727	0.1711	
RCC	0.1858	0.1867	0.1768	0.1480	0.1502

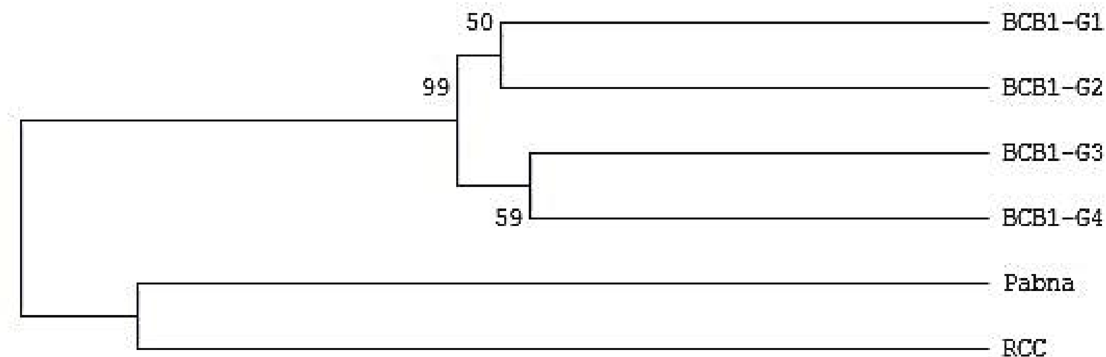


Figure 1. Dendrogram showing genetic relationship among four generations of BCB-1, Pabna cattle and RCC.

distance with all the four generation of BCB-1 but lower with Pabna Cattle.

The Dendrogram formed based on DA genetic distance showed that all the four generations of BCB-1 grouped together. BCB-1 generations are separated from Pabna cattle and RCC. Pabna cattle and RCC population produced separate cluster (Figure 1).

Cluster 1 consisted of 1st and 2nd generations and cluster 2 consisted of 3rd and 4th generation of BCB-1. All generations of BCB-1 of Cluster 1 and 2 grouped together and separated from the Pabna cattle and reference RCC population.

Because of applying pure breeding program in BCB-1, MNA and heterozygosity decreased in subsequent generations. These finding was supported by Ma *et al*. (2019). Because their findings provided a novel understanding about genome changes due to artificial selection and their impact on fertility and immunity genes to reverse the declines in fertility and immunity in Holstein cattle. The BCB-1 cattle in 3rd and 4th generation becoming more homogeneous. On the other hand, BCB-1 showed more genetic distance from its predecessor (Pabna cattle) and also differed in MNA and heterozygosity value.

Conclusion

This genetic study revealed that four generations of BCB-1 grouped together and separated from their predecessor (Pabna cattle) and reference RCC. Homozygosity has increased in subsequent generation of BCB-1. Therefore, it could be evident that BCB-1 is a unique cattle population of Bangladesh.

References

Bhuiyan, M.S.A., Hanotte, O. and Bhuiyan, A.K.F.H. 2005. Genetic diversity of Red Chittagong Cattle of Bangladesh using microsatellite markers. Progress. Agric. 16(1).: 141-148.

Bhuiyan, M.S.A., Bhuiyan, A.K.F.H., Yoon, D.H., Jeon, J.T., Park, C.S. and Lee, J.H. 2007. Mitochondrial DNA Diversity and Origin of

Red Chittagong Cattle. Asian-Aust. J. Anim. Sci. 20(10): 1478-1484.

Bhuiyan, A.K.F.H. 2007. Cattle and livelihood in Bangladesh. Article from web resource. http://www.vishwagou.org/ date 02/09/2008.

Deb, G.K., Mufti, M.M.R., Mostari, M.P. and Huque, K.S. 2009. Reproductive efficiency of BLRI Cattle Breed-1 at farm condition. Bang. J. of Progress. Agric. 19(2): 85-92.

Deb, G.K., Mufti, M.M.R., Mostari, M.P. and Huque, K.S. 2008. Genetic Evaluation of BLRI Cattle Breed-1: heritability & genetic correlation. Bang. J. of Anim. Sci. 37: 22-28.

Felsenstein, J. 1995. PLYLIP phylogeny software version 3.7c. Department of Genetics, Uniersity of Washington, Seattle, Washington.

Habib, M.A. Bhuiyan, A.K.F.H. and Amin, M.R. 2009. Birth weight and its non-genetic effect in Red Chittagong Cattle (RCC) in a closed nucleus herd. Int. J. Bio. Res. 1(1):35-39.

Habib, M.A. Bhuiyan, A.K.F.H. and Amin, M.R. 2012. Genetic and phenotypic parameter on fertility traits of Red Chittagong Cattle in Bangladesh. The Bang. Vet. 29(2): 78–89.

Hossain, S.M.J., Afroz, M.F., Bhuiyan, A.K.F.H. and Habib, M.A. 2018. Milk production performance of Red Chittagong Cattle based on phenotypic and genetic parameters. Glo. Adv. Res. J. Agric. Sci. 7(4): 110-117.

Ma, L. I., Sonstegard, T. S., Cole, J. B., VanTassell, C. P, Wiggans, G. R., Crooker, B. A. Cheng, T Prakapenka, D., Liu, G. E and Da, Y. 2019 . Genome changes due to artificial selection in U.S. Holstein cattle. BMC Genomics. 20:128.

Minch, E., Ruiz-Linares, A. and Goldstein, D.B. 1995. Microsat (version 1.5d): a program for calculating statistics on microsatellite allele data.

Ota, T. 1993. DISPAN: Genetic distance and phylogenetic Analysis. Pennsylvania state university, University Park, PA.