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A Study on β-Casein Gene Polymorphism in Crossbred Cattle and Murrah / Graded Murrah Buffalo in Tamil Nadu

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ABSTRACT

Background: Majority of the people believed that, only the native breeds have A2 milk than exotic or crossbred animals. There is no enough research on milk protein variants have been carried out in Indian zebu cattle. Present study was conducted to screen more number of crossbred cattles and buffaloes in Tamil Nadu to identify the frequency of A1 and A2 alleles in the population.

Methods: The study was conducted on 68 cattle and 172 murrah/graded murrah buffaloes to explore the polymorphic variants of β-casein gene. Genomic DNA was extracted by phenol-chloroform method. Polymerase chain reaction (PCR) was performed with allele specific primers to amplify a 244 bp long fragment of beta-casein gene and visualized in 2% agarose gel electrophoresis. The population genetic indices were calculated based on the formulas.

Result: In the present study revealed higher level of A2A2 genotype frequency (1.00) and fixation of A_2 allele in kangayam cattle and murrah/graded murrah buffalo. The observed frequency of A1A1, A1A2 and A2A2 genotypes were 0.38,0.62 and 0.00 for jersey crossbred and 0.29, 0.71 and 0.00 for HF crossbred cattle. The range of Expected homozygosity (0.50 to 1.00), polymorphism information content (0.30 to 0.38), effective number of alleles (1.00 to 2.00) and level of possible variability realization value (44.54% to 100%) reflected existence of medium genetic variability in studied population.

Key words: Allele specific PCR, Buffalo, β-casein gene polymorphism, Cattle.

INTRODUCTION

In the recent decades have we much heard about the popularity of A2 milk, which is believed to have more nutritious with no health hazards than A1 milk. Among the three variants of casein, which is the major milk protein, β-casein is the second common type with 13 allelic variants (Farrel *et al*., 2004). About 25-30 per cent casein in the milk is beta-casein-the protein that differentiates A1 from A2 milk. The point mutation in the beta-casein gene present in the 6th chromosome changes proline (A2 β-casein) to histidine (A1 β-casein) at 67th position of the resultant amino acid chain.

A2 beta casein has been found to be associated with reduced serum cholesterol and decrease concentration of low density lipoprotein which play an important role in prevention of a wide range of human vascular diseases (Ikonen *et al*., 2001 and Meisel and Fitzgerald, 2003). Thus formed BCM-7 get absorbed through small intestine and in long run believed to lead to type-I diabetes, arteriosclerosis, ischemic heart disease, schizophrenia and autism (Bell *et al*., 2006 and Truswell, 2005). Even though A2 milk also yields beta-casomorphin it is not a 7 aminoacid compound instead is a 9 aminoacid compound. This has been not proved to have harmful effects on human health (Boltstein *et al*., 1980).

European population is suffering from Ischemic heart disease which was linked to their intake of specific milk protein. But Masai and Samburu of Africa have never reported any heart diseases despite of their animal milk rich diet (McLachlan, 2001). The genetic characterisation of most of the European cattle breeds revealed their milk type as

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A1 except for Jersey and Guernsey. Most of the *Bos indicus* breeds are proved to be A2 type milkers (Mishra *et al.,* 2009). By comparing both the above mentioned findings an association between milk type and health conditions were identified. A1 milk consumers exhibited more health associated problems than A2 consumers. Another reason for the acceptance of A2 milk is its calcium content. Calcium intake from milk can reduce osteoporosis, colon cancer and to an extent reduce weight gain. The ideal calcium to magnesium ratio in the human body should be 2:1 whereas A1 milk has a higher ratio in the range 10:1. This can lead to magnesium deficiency in humans (Boro *et al*., 2016).

There is no much evidence on the bovine beta-casein variants in Tamil Nadu bovine population. The present study was conducted to analyse the frequency of the A1 and A2 allele of beta-casein gene in crossbred cattle and buffalo of Tamil Nadu.

MATERIALS AND METHODS

Experimental animals

A total of 68 cattle and 172 Murrah/graded Murrah buffaloes were included in this study (Table 1) and research work was performed at Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal from August, 2016 to June, 2017. Blood samples were collected in EDTA coated vacutainer by venipuncture of the jugular vein following the guidelines of MoDAD (Measurement of Domestic Animal Diversity, Rome).

DNA extraction

The standard phenol-chloroform method (Sambrook and Russel, 2001) was followed to extract the genomic DNA. The concentration and purity of the DNA samples were checked using nanodrop. All the samples showing an $OD_{260/280}$ ratio between 1.8 and 2.0 (absence of protein and RNA contamination) were used for further analysis.

Polymerase chain reaction (PCR)

Allele specific primers were used to amplify a 244 bp long fragment of beta-casein gene. Two forward primers differing in a single nucleotide and a common reverse primer were used for independent amplification of A1 and A2 alleles (Ganguly *et al*., 2013a). The forward primers used for amplification were, one with 'A' as the final nucleotide (IGBhF 5′ CTT CCC TGG GCC CATCCA 3′) and other with 'C' as the end nucleotide (IGBpF 5′ CTT CCC TGG GCCCAT CCC 3′), of which former with the common reverse primer amplified histidine specific amplicon whereas latter amplified proline specific amplicon. The common reverse primer used was (IGBR 5′ AGA CTG GAG CAGAGG CAG AG 3′). The polymerase chain reaction was performed with a total volume of 25 µl, with 12.5 µl of Ampliqon Taq DNA Polymerase Master Mix red (2X), 0.5 µl each of forward and reverse primers, 10 µl nuclease free water and 1.5 µl of DNA under following thermal conditions: Initial denaturation at 94°C for 5 min, followed by 5 cycles of 94°C for 30 sec, 64°C for 30 sec and 72°C for 30 sec; there after 30 cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 30 sec and a final extension of 72°C for 5 min. PCR ampliqons were visualized after electrophoresis in 2% agarose gel and the images were documented in a gel documentation system (Bio-Rad Gel Doc™).

Statistical analysis

The gene and genotype frequencies were calculated by simple frequency calculations (Falconer and Mackay, 1996). The deviation of genotypic frequencies from expectations (Hardy-Weinberg equilibrium) was analyzed using Chi-square test of significance as described by Snedecor and Cochran (1980). The population genetic indices were evaluated by following parameters.

Effective number of alleles (ENA)

Effective number of alleles (Ne) was calculated as per the formula given by Kimura and Crow (1964).

$$Ne = \frac{1}{\sum Pi^2}$$

Where,

$$Pi^2 = p^2+q^2$$

Experimental heterozygosity (He) (Nei, 1978)

Heterozygosity is the state of possessing different alleles at a given locus in regard to a given character. It is a measure of heterozygosity or genetic variation in a population. The population heterozygosity at a locus is given by formula:

$$E = \sum Pi^2$$

Expected heterozygosity

The expected heterozygosity per locus (E) is defined as the mean of heterozygosity over all structural loci in the genome.

$$H_e = 1 - \sum P_i^2$$

However,
the unbiased estimate of expected heterozygosity at a locus is (if N <50): N is the sample size:

$$H_e = \frac{2N}{2N - 1}\left[1 - \sum_{i=1}^{n} Pi^2\right]$$

Polymorphism information content (PIC)

The polymorphism information content (PIC) was calculated using the individual frequencies of the alleles occurred at each locus (Botstein *et al*., 1980) using the following formula:

$$PIC = \left[1 - \left\{\sum_{i=1}^{K} X_i^2 - \sum_{i=1}^{K-1}\sum_{j=1}^{K} 2X_i^2 X_j^2\right\}\right]$$

Level of possible variability realization (V%) (Kimura and Crow, 1970)

$$V = \frac{1 - E}{1 - 1/N} \times 100$$

RESULTS AND DISCUSSION

The isolated DNA was quantified and checked for quality in Nanodrop. The mean yield of DNA isolated was 439.56 ng/µl. The ratios of optical densities were around 1.8 indicating

good deproteinisation. The PCR product (244 bp) of beta casein gene in cattle and buffaloes were analyzed in 2 per cent agarose gel (Fig 1 and 2). The allele and genotype frequencies were calculated. Genetic variants of beta casein gene observed in studied population are shown in Fig 1 and 2. The genotypic frequencies and allelic frequencies of A1A2 genetic variants in cattle and buffalo are presented in Table 2. The tested populations of kangayam cattle and murrah/graded murrah buffaloes were monomorphic, whereas Jersey and Holstein Friesian crossbred cattle at β-casein gene locus were found to be polymorphic. A2A2 genotype alone noticed in kangayam cattle and murrah/ graded murrah buffaloes not a single animal had A1A1 and A1A2 genotypes. But crossbred cattle had A1A1, A1A2 and A2A2 genotypes in studied population.

The frequency of A1A1, A1A2 and A2A2 genotypes were 0.00, 0.00 and 1.00 for kangayam cattle and graded murrah buffaloes respectively in tested population. The frequency of A1A1, A1A2 and A2A2 genotypes were 0.38, 0.62 and 0.00 for jersey crossbred and 0.29, 0.71 and 0.00 for HF crossbred cattle at farmer's herd. In Livestock Farm Complex, Jersey crossbred had frequency of 0.18, 0.29 and 0.53 for A1A1, A1A2 and A2A2 genotype respectively. The allelic frequency of A1 and A2 was 0.69 and 0.31 for jersey crossbred and 0.64 and 0.36 for HF crossbred cattle at farmer's herd. The genotype frequency was ranged between 0.00-0.38, 0.00-1.00 and 0.00-1.00 for A1A1, A1A2 and A2A2 respectively among all the breeds under study. Heterozygotes were higher in number than homozygotes in Jersey and HF crossbred as expected. All the studied population was under Hardy Weinberg equilibrium which was confirmed using chi-square test ($p>0.5$).

It was observed that A2 allele was fixed in Kangayam breed of cattle and all buffaloes. Similar to our findings, Pandey *et al*., (2021) reported only the presence of A2 allele in Malvi and Nimari indigenous cattle breeds. The fixation of A2 allele in riverine buffaloes (Mishra *et al*., 2009) could be confirmed from the present data. Uniformly Malarmathi *et al*., (2014) also found high frequency of A2 allele in Kangayam breed of cattle and A1 allele in crossbred cattle of Tamil Nadu. Similar allele frequency was observed in Ongole (0.94), Frieswal heifer (0.68) and Frieswal bulls (0.56) in earlier studies (Ganguly *et al.,* 2013a). Various study on different indigenous cattle breeds showed high frequency of A2 allele and particular allele fixation (Ganguly *et al*., 2013a, Kumar *et al*., 2019, Mishra *et al*., 2009 and Ramesha *et al*., 2016).

In this study, A1 allele frequency of jersey and HF crossbred cattle was 0.69 and 0.64 respectively.

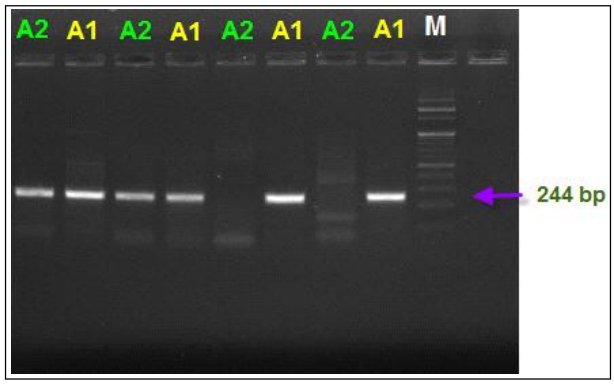

Fig 1: Allele specific PCR products of beta casein genotypes in cattle. Lane 1,2,3 and 4= A1A2 genotype, Lane 5,6,7 and 8= A1A1 genotype, M= 100 bp DNA Marker.
Note: Band noticed in A1 and A2 lane resulted A1A2 genotype, A1 alone resulted A1A1 genotype and A2 alone resulted A2A2 genotype.

Table 1: Details on number of blood samples collected from different location.

Location of farm	Breed	No. of samples
Cattle		
Livestock Farm Complex (LFC), Veterinary College and Research Institute, Namakkal, Tamil Nadu.	Jersey cross bred cow	17
Department of Veterinary Obstetrics and Gynaecology, Veterinary College and Research Institute, Namakkal, Tamil Nadu.	Kangayam bull	04
	Jersey bull	02
	Holstein Fressian bull	02
Farmers Herd, Namakkal, Tamil Nadu	Jersey cross bred cow	29
	Holstein Fressian cross bred cow	14
	Total	68
Buffalo		
Post Graduate Research Institute in Animal Science (TANUVAS), Kattupakkam, Tamil Nadu.	Murrah/	30
Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District Andhra Pradesh	Graded Murrah	70
Saraswathi Krishi Vigyan Kendra, Karur district, Tamil Nadu.		19
Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu.		20
Farmers Herd, Namakkal, Tamil Nadu		33
	Total	172

Predominant of A1 allele frequency was reported in numerous crossbred cattle by many researchers (Ganguly *et al*., 2013b; Muhammed and Stephens, 2012; Bech and Kristainsen, 1990; Ehrmann *et al*., 1997; Ikonen *et al*., 1997 and Hanusová *et al*., 2010) but less frequency of A1 allele was noticed crossbred by (Ganguly *et al*., 2013a; Kaminski *et al*., 2007; Malarmathi *et al*., 2014; Kaminski *et al*., 2006; Winkelmen and Wickham, 1997 and Manga *et al*., 2006).

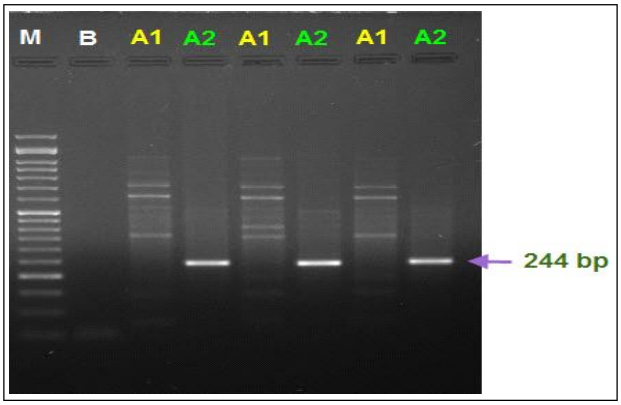

Fig 2: Allele specific PCR products showing the A2A2 genotypes of beta casein in buffaloes. M= 50 bp DNA Marker, B= Blank.

In HF bull, effective number of alleles was two indicating that both A1 and A2 alleles were present in the population. The frequency of A2 allele was observed to be higher than A1 in the samples from LFC. On the contrary the exotic breeds like HF and Jersey which are recommended for crossbreeding in India had a higher range of A1 allele (0.01 to 0.72) frequency all over the globe (Kaminski *et al.,* 2007). The predominance of A2 allele was identified in Carora cattle population (Caroli *et al.,* 2009); Simmental breed and Holstein breed (Miluchova *et al.,* 2014).

The value of theoretical heterozygosity (He), observed heterozygosity (Hobs), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA) and level of possible variability realization (V%) were listed in Table 3. Observed heterozygosity was highest in HF bull (0.5) and lowest in Jersey (0.38). Expected heterozygosity was highest in HF bull (0.67) and lowest in Jersey breed of cattle (0.44). Polymorphism information content (PIC) value is an indication of degree of informativeness of a marker and genetic diversity of the population. The value of PIC ranged between 0.30 to 0.38 indicating medium level of genetic diversity in studied population. Similarly, medium and low level of PIC values were observed in Frieswal and Tharparkar breeds

Table 2: Genotype and allele frequencies of A1 and A2 variants in different cattle and buffalo breeds.

Source	Genotype frequency			Allele frequency		P value
	A1A1	A1A2	A2A2	A1	A2	
Jersey CB at LFC	0.18	0.29	0.53	0.32	0.68	0.393
	(3)	(5)	(9)			
Kangayam bull	0.00	0.00	1.00	0.00	1.00	-
	(0)	(0)	(4)			
Jersey bull	0.00	0.50	0.50	0.25	0.75	0.894
	(0)	(1)	(1)			
HF bull	0.00	1.00	0.00	0.50	0.50	0.368
	(0)	(2)	(0)			
Jersey CB at farmers herd	0.38	0.62	0.00	0.69	0.31	0.053
	(11)	(18)	(0)			
HF CB at farmers herd	0.29	0.71	0.00	0.64	0.36	0.117
	(4)	(10)	(0)			
Buffalo	0.00	0.00	1.00	0.00	1.00	-
	(0)	(0)	(172)			

Table 3: Effectiveness of allele in different cattle and buffalo breeds.

Breed/location	H_{obs}	H_e	PIC	E	ENA	V%
Jersey CB at LFC	0.44	0.45	0.34	0.56	1.77	46.75
Kangayam bull	-	-	-	1.00	1.00	-
Jersey bull	0.38	0.50	0.30	0.62	1.60	76.00
HF bull	0.50	0.67	0.38	0.50	2.00	100.00
Jersey CB at farmers herd	0.43	0.44	0.34	0.57	1.75	44.54
HF CB at farmers herd	0.46	0.48	0.35	0.54	1.85	49.54
Buffalo	-	-	-	1.00	1.00	-

Heterozygosity (H_e); Observed heterozygosity (H_{obs}); Polymorphism information content (PIC); Expected homozygosity (E); Effective number of alleles (ENA) and level of possible variability realization (V%).

(0.3575 and 0.0739) at cattle and buffalo farm, Izatnagar (Kumar *et al*., 2020). Kumar *et al*. (2019) reported that medium level of PIC value (0.3515) in Vrindavani and low level (0.1064) in Sahiwal cattle. The range of expected homozygosity, effective number of alleles and level of possible variability realization values were 0.50 to 1.00, 1.00 to 2.00 and 44.54% to 100% respectively in the screened population. Similar to our findings, Kumar *et al*., (2020) observed same level of effective allele number (1.87) in Tharparkar and Frieswal population.

It is of great advantage to humans, if the uncertainty over A1/A2 variants finds a solution. As a first step it is necessary to screen all the crossbred cattle population of India which are the major contributor of milk to the economy to identify the A1 variant. An appropriate strategic breeding might be essential to tackle the present field scenario.

CONCLUSION

From this study, we can conclude that the A2 allele is fixed in buffalo population and has a higher frequency in native Indian cattle breeds. Crossbred cattle have comparatively lower frequency of A2 allele. This economically important feature can be exploited in planning cattle breeding programmes so as to increase the frequency of A2 allele in crossbred cattle population.

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