RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 1: 24-28 (January 2023)

Detection of A1 and A2 Alleles at Beta-casein Locus in Bargur and Umblachery (Indian Zebu) Cattle Breeds by Allele-specific PCR

A. Raja[1], R. Rajendran[2], P. Ganapathi[3]

10.18805/IJAR.B-4273

ABSTRACT

Background: Many genetic variants of beta-casein in different breeds of cattle have been reported. The A1 and A2 are the most common variants. The breeds of Zebu cattle have high frequency of A2 allele or monomorphic for A2 allele. The current study aimed to screen Indian Zebu cattle breeds, Bargur and Umblachery, for A1 and A2 alleles at beta-casein locus.

Methods: A total of 48 Bargur and 42 Umblachery cattle were genotyped for β-casein (CSN2) gene using allele-specific PCR. The gene and genotype frequencies were estimated. The theoretical heterozygosity (He_{exp}), experimental heterozygosity (He_{obs}), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA) and level of possible variability realization (V%) were calculated.

Result: The investigation revealed the presence of both A1 and A2 alleles at beta-casein locus in both Bargur and Umblachery cattle breeds. The A1A1 genotype was not observed in both the breeds. The frequencies of A1A2 and A2A2 genotypes were 0.125 and 0.875 respectively in Bargur and 0.050 and 0.950 respectively in Umblachery breed. The study indicated the predominance of A2 variant in both the breeds. The frequencies of A1 and A2 alleles were 0.063 and 0.937 respectively in Bargur and 0.02 and 0.98 respectively in Umblachery breed. The values of experimental heterozygosity (He_{obs}), theoretical heterozygosity (He_{exp}), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA), level of possible variability realization (V%) were 0.125, 0.1163, 0.1095, 0.8837, 1.131 and 11.88 respectively in Bargur breed. These values were 0.048, 0.0468, 0.0458, 0.9532, 1.049 and 4.79 respectively in Umblachery population. The observed heterozygosity and PIC values revealed the existence of very low genetic variability in the tested populations. The present work will be a contribution to the study on beta-casein locus in Indian zebu cattle.

Key words: A2 allele, Bargur, β-casein gene, Indian cattle, PCR, Polymorphism, Umblachery.

INTRODUCTION

Caseins and whey proteins are the major proteins of bovine milk. Among these, caseins account for 80% of milk proteins which is secreted by cells of the mammary glands (Sulimova *et al*., 2007). Among casein proteins, beta-casein constitutes about 25 - 30% of total milk proteins and comprises of 209 amino acids with a molecular weight of 24 kD (Keating *et al*., 2008). Bovine casein milk proteins are synthesized by four genes, alpha s1, beta, alpha s2 and kappa (*CSN1S1*, *CSN2*, *CSN1S2* and *CSN3* respectively) (Farrell *et al*., 2004). The four casein genes were mapped to bovine chromosome 6 in the order of *CSN1S1-CSN2-CSN1S2-CSN3* (Ferretti *et al*., 1990; Threadgill and Womack, 1990).

Many genetic variants of beta-casein in different breeds of cattle have been reported including A1, A2, A3, B, C, D, E, F, G, H1, H2 and I. The A1 and A2 are the most common variants, B variant is less common and A3 and C variants are rare (Jaiswal *et al*., 2014; Sharma *et al*., 2013). Each variant is differing from other variants in amino acid substitution at a fixed position. In polypeptide chain position 67, His is substituted by Pro, for A1 and A2, respectively.

It was shown that A1 beta casein produces betacasomorphin-7 or BMC-7 during the digestion of raw or processed milk or cheese in the human gut and BMC-7 may be a significant riskfactor in human ischemic heart disease, arteriosclerosis, type I diabetes and sudden infant death syndrome (Elliot *et al*., 1999; Thorsdottir *et al*., 2000; McLachlan, 2001; Sun *et al*., 2003; Tailford, 2003).

[1]Education Cell, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal-637 002, Tamil Nadu, India.
[2]Laboratory Animal Medicine Unit, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai-600 051, Tamil Nadu, India.
[3]Bargur Cattle Research Station, Bargur, Anthiyur Taluk, Erode-638 501, Tamil Nadu, India.

Corresponding Author: R. Rajendran, Laboratory Animal Medicine Unit, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai-600 051, Tamil Nadu, India.
Email: rajendran.r@tanuvas.ac.in

How to cite this article: Raja, A., Rajendran, R. and Ganapathi, P. (2023). Detection of A1 and A2 Alleles at Beta-casein Locus in Bargur and Umblachery (Indian Zebu) Cattle Breeds by Allele-specific PCR. Indian Journal of Animal Research. 57(1): 24-28. doi: 10.18805/IJAR.B-4273.

Submitted: 04-08-2020 **Accepted:** 13-01-2021 **Online:** 09-03-2021

Lipkin *et al*., (2008) observed negative influences of A1 variant on production traits. Instead; it was shown that A2 variant has positive relationship with milk performance traits such as milk protein and milk yield apart from health promoting properties (Ikonen *et al*., 1999; Ikonen *et al*., 2001; Olenski *et al*., 2010). Moreover, the level of BCM-7 in beta-casein A1 hydrolysed milk is reported to be four times higher than the A2 milk (Cieslinska *et al*., 2007). However, the overall evidence for gastrointestinal effects from A1 and BCM-7 in animal and *in vitro* studies is conclusive, but the evidence from human studies is still limited and the clinical implication of A1 milk on human health is still under discussion.

An attempt was made to study the frequency of A1 and A2 alleles at beta-casein locus in Bargur and Umblachery cattle of Tamil Nadu, India. This is the first report on the status of the beta-casein alleles in these breeds. Bargur cattle are medium sized animals; they are usually red coloured with white patches. These are bred in the Bargur hills of Western Ghats in Erode district of Tamil Nadu (Ganapathi *et al*., 2009). Umblachery animals are short and grey coloured. These are bred in Thiruvarur and Nagapattinam districts of Tamil Nadu (Rajendran *et al*., 2008).

MATERIALS AND METHODS

This study was carried out on Bargur and Umblachery breeds of cattle of Tamil Nadu, India. The blood samples were collected from the randomly selected animals in the home tract of respective breeds of cattle. In the present study, a total of 48 Bargur and 42 Umblachery animals were screened for A1 and A2 alleleic variants in bovine beta-casein (CSN2) gene.

Sample collection and DNA extraction

Blood sample (5-10 ml) was aseptically collected from each animal in an EDTA containing vacutainer tube by jugular vein puncture and transported to laboratory at 4°C. Genomic DNA was isolated from the blood using standard phenol chloroform extraction method. Nanodrop (Thermoscientific) was used to assess the quality and quantity of DNA. Samples showing an optical density ratio (260 nm/280 nm) of between 1.8-2.0 were diluted to 50 ng/µl and stored at -20°C.

Allele specific PCR

Allele specific PCR (AS-PCR) was carried out as reported by Ganguly *et al*. (2013). AS-PCR was carried by using the forward primers, IGBhF (5′-CTTCCCTGGGCCCATCCA-3′), IGBpF (5′-CTTCCCTGGGCCGATCCC-3′) and common reverse primer, IGBR (5′-AGACTGGAGCAGAGGCAGAG-3′). For each sample, two separate reactions were set with primer pairs, IGBhF and IGBR to amplify the 244 bp A1 variant (CCA-Histidine) and primer pairs IGBpF and IGBR were used to amplify the 244 bp A2 variant (CCC - Proline). AS-PCR was carried out in a total volume of 20 µl contained 50 ng of DNA template, 10 µl Taq DNA polymerase 2x master mix (Ampliqon), 1 µl each of forward and reverse primers (10 pmoles each) and nuclease free water to final volume of 20 µl. PCR was carried out in a T100 thermal cycler (BioRad) with cyclic conditions of 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; thereafter 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 products were visualized after electrophoresis in a 2% agarose gel (Fig 1).

The gene and genotype frequencies were calculated as per Falconer (1991). Effectiveness of allele incidence was evaluated using theoretical heterozygosity (He_{exp}) as per Nei (1973), experimental heterozygosity (He_{obs}), expected homozygosity (E), effective number of alleles (ENA), level of possible variability realization (V%). as per Crow and Kimura (1970). Polymorphism information content value (PIC) was estimated according to Botstein *et al*. (1980).

RESULTS AND DISCUSSION

The genotype and gene frequencies of beta-casein A1A2 in Bargur and Umblachery cattle are presented in Table 1. Only two types of genotypes *i.e*. A1A2 and A2A2 were observed in both the breeds (Fig 1). We detected a predominance of the A2A2 genotype; a lower frequency of the A1A2 genotype and absence of the A1A1 genotype.

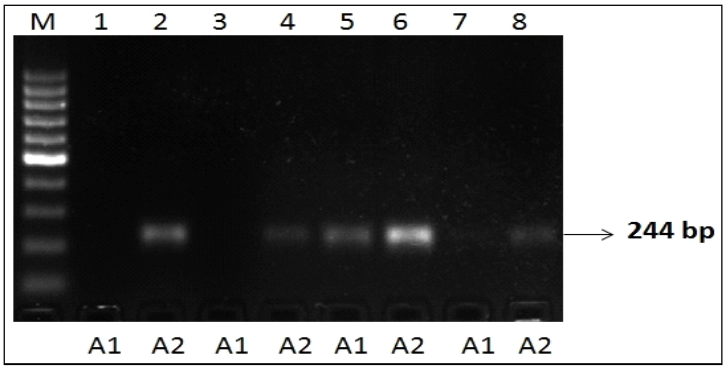

Fig 1: Agarose gel electrophoresis of AS-PCR products of beta-casein genotypes with 244 bp. Lane M: Marker (100 bp DNA ladder). Lane 1, 3, 5, 7-A1 specific PCR. Lane 2, 4, 6, 8-A2 specific PCR. Lanes 1 and 2 demonstrate A2A2 genotype; lanes 3 and 4 indicate A2A2 genotype; lanes 5 and 6 show A1A2 genotype; lanes 7 and 8 shows A2A2 genotype.

Among the 48 Bargur animals screened, 42 animals were of A2A2 genotype and the 6 animals were of A1A2 genotype. The genotype frequencies of A1A1, A1A2 and A2A2 were 0.000, 0.125 and 0.875 respectively in Bargur breed. The frequency of allele A2 was very high in the population and amounted to 0.937. The frequency of allele A1 was 0.063 in Bargur breed.

Out of 42 Umblachery cattle, 40 animals were of A2A2 genotype and 2 animals were having A1A2 genotype (Fig 1). The genotype frequencies of A1A2 and A2A2 were 0.048 and 0.952 respectively in Umblachery breed. The frequencies of A1 and A2 alleles were 0.024 and 0.976 respectively in Umblachery breed. Hence, this study revealed the presence of A1 allele in low frequency in purebred Umblachery and Bargur cattle. The present results showed predominance of A2A2 genotype and A2 allele in both the breeds. The results of our study are compatible with the results of Mishra *et al.* (2009) who studied the β-casein variants among 15 Indian cattle breeds and reported the frequencies of A2 allele as 0.904 in Malnad Gidda, 0.891 in Kherigarh and fixation of allele in Kangayam, Nimari, Red Kandhari, Malvi, Amritmahal, Kankrej, Gir, Sahiwal, Hariana, Tharparkar, Rathi, Mewathi and Red Sindhi. They reported the absence of A1A1 genotype among the investigated breeds and also near fixation of A2 allele in Indian cattle breeds except for very low frequency of A1A2 in Malnad Gidda and Kherigarh cattle. They have further estimated the mean frequencies of A1 and A2 alleles among these 15 breeds as 0.013 and 0.987 respectively. The predominance of A2 allele had also been reported in Sahiwal, *i.e.*, 0.93 in Sahiwal (Mir *et al*., 2014) and 0.94 in Ongole (Ganguly *et al*., 2013). Malarmathi *et al*. (2014) did not find any A1 allele and reported that A2 allele had been fixed in Kangayam breed of cattle, one of the four recognised breeds of cattle of Tamil Nadu state of India. Ramesha *et al*. (2016) screened the various breeds of cattle of India and reported the frequencies of A2 allele as 0.986 in Malnad Gidda, 0.958 in Kasargod cattle and 1 (fixed) in Deoni and Khillar cattle breeds. Kumar *et al*. (2018) also reported the abundancy of A2 allele in Sahiwal cattle and the frequencies of A1 and A2 alleles were 0.06 and 0.94 respectively with absence of A1A1 genotype in the population they studied. Genotype frequencies for A1A2 and A2A2 genotypes were 0.13 and 0.87 in Sahiwal cattle. The frequency of A1 allele in Bargur and Umblachery breeds is in contrast to the allelic frequency of A1 allele, ranging from 0.01 to 0.72 throughout the world (Kaminsky *et al*., 2006). If the beneficial property of the A2 milk is proved beyond the doubt, it can be commercially exploited and utilised for conservation of Bargur cattle, which has become endangered (Ganapathi *et al*., 2009) and Umblachery cattle, the population size of which is declining at a faster rate.

Effectiveness of alleles

The values of experimental heterozygosity (H_{obs}), theoretical heterozygosity (H_{exp}), polymorphism information content (PIC), expected homozygosity (E), effective number of alleles (ENA), level of possible variability realization (V%) were 0.125, 0.1163, 0.1095,0.8837, 1.131 and 11.88 respectively in Bargur population. These values were 0.048, 0.0468, 0.0458, 0.9532, 1.049 and 4.79 respectively in Umblachery population (Table 2). In the Bargur cattle, the bovine *CSN2* gene showed a high proportion of A2A2 ho-mozygosity (87.5%), as described with the high value of the coefficient of homozygosity (0.8837). In the Umblachery cattle, the same locus showed a highest proportion of A2A2 homozygosity (95.2%) as described with the highest value of coefficient of homozygosity (0.9532). Effectiveness of alleles in a population can be described with the effective number of alleles. In a bi-allelic system, a limit of 2.0 indicates that both alleles are effectively involved in the development of genotypes. These results revealed that both the breeds have lower amount of heterozygosity. In this study, the ENA decreased to 1.131 in Bargur and 1.049 in Umblachery cattle, showing that the effect of alleles A1 and A2 is not balanced in both the breeds. Polymorphism information content, an indicative of degree of informativeness of a marker and genetic diversity ranges from 0 to 1. In present investigation, the PIC values (0.1095 and 0.0458) were substantially lower than a threshold value (0.5), also indicating a low polymorphic level and thereby low genetic diversity. The low level of polymorphism caused a decrease in a level of possible variability realization (11.88 and 4.79%).

Table 1: Genotype and gene frequencies of beta-casein (CSN2) gene in Bargur and Umblachery cattle.

Breed	No. of animals	Genotype frequency			Gene frequency	
		A1A1	A1A2	A2A2	A1	A2
Bargur	48	0.00 (n=0)	0.125 (n=6)	0.875 (n=42)	0.062	0.938
Umblachery	42	0.000 (n=0)	0.048 (n=2)	0.952 (n=40)	0.024	0.976

Table 2: Effectiveness of alleles for beta-casein (CSN2) gene in Bargur and Umblachery cattle.

Breed	H_{obs}	H_{exp}	PIC	E	ENA	V%
Bargur	0.125	0.1163	0.1095	0.8837	1.131	11.88
Umblachery	0.048	0.0468	0.0458	0.9532	1.049	4.79

CONCLUSION

The study revealed the presence of A1 allele in low frequency in purebred Bargur and Umblachery cattle. Hence, it is reiterated that all the Zebu cattle has A2 variant only is a myth. Still considerable uncertainty prevails with the issue of A1/A2 milk. Although, the clinical implications of A1 milk on human health is still under discussion, it will be necessary as a precaution to screen the bulls to prevent the spread of A1 allele in the cattle population. Since CSN2 genotyping is based on DNA, there is a possibility of decreasing the frequency of A1 allele by the screening of bulls. However, the screening is possible only for the bulls used in AI programme. The screening should be carried out for the bulls of indigenous breeds of cattle in AI programme also. The genotyping of Indian zebu cows for CSN2 gene should be obligatory when the milk is sold as A2 milk.

REFERENCES

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. American Journal of Human Genetics. 32: 314-331.

Crow, J.F. and Kimura, M. (1970). An Introduction to Population Genetics Theory. Burgess Publishing, Minneapolis.

Cieslinska, A., Kaminski, S., Kostyra, E. and Sienkiewicz-Szlapka, E. (2007). Beta-casomorphin 7 in raw and hydrolyzed milk derived from cows of alternative b-casein genotypes. Milchwissenschaft. 62: 125-127.

Elliot, R.B., Harris, D.P., Hill, J.P., Bibby, N.J. and Wasmuth, H.E. (1999). Type I (insulin dependent) diabetes mellitus and cow milk: Casein variant consumption. Diabetologia. 42: 292-296.

Farrell Jr, H.M., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K., Hicks, C.L., Hollar, C.M., Ng-Kwai-Hang, K.F. and Swaisgood, H.E. (2004). Nomenclature of the proteins of cows' milk. Sixth revision. Journal of Dairy Science. 87: 1641-1674.

Ferretti, L., Leone, P. and Sgaramella, V. (1990). Long range restriction analysis of the bovine casein genes. Nucleic Acids Research. 18: 6829-6833.

Ganapathi, P., Rajendran, R. and Subramanian, A. (2009). Distribution and population status of Bargur cattle. Indian Veterinary Journal. 86: 971-972.

Ganguly, I., Gaur, G.K., Umesh, S., Sunil, K. and Sandeep, M. (2013). Beta-casein (CSN2) polymorphism in Ongole (Indian Zebu) and (HF × Sahiwal crossbred) cattle. Indian Journal of Biotechnology. 12: 195-198.

Ikonen, T., Bovenhuis, H., Ojala, M., Ruottinen, O. and Georges, M. (2001). Association between casein haplotypes and first lactation milk production traits in Finnish Ayrshire cows. Journal of Dairy Science. 84: 507-514.

Ikonen, T., Ojala, M. and Ruottinen, O. (1999). Association between milk protein polymorphisms and first lactation milk production traits in Finnish Ayrshire cows. Journal of Dairy Science. 82: 1026-1033.

Jaiswal, K., De, S. and Sarsavan, A. (2014). Detection of single nucleotide polymorphism by T-ARMS PCR of cross bred cattle Karan Fries for A1, A2 beta casein types. International Journal of Scientific Research in Biological Science. 1: 18-22.

Kaminski, S., Rusc, A. and Cieslinska, A. (2006). A note on frequency of A1 and A2 variants of bovine beta-casein locus in Polish Holstein bulls. Journal of Animal and Feed Sciences. 15: 195-198.

Keating, A., Smith, T., Ross, R. and Cairns, M. (2008). A note on the evaluation of a beta-casein variant in bovine breeds by allele-specific PCR and relevance to β-casomorphin. Irish Journal of Agricultural and Food Research. 47: 99-104.

Kumar, S., Singh, R.V. and Chauhan, A. (2018). Molecular charac-terization of A1/A2 Beta-casein Alleles in Vrindavani crossbred and Sahiwal cattle. Indian Journal of Animal Research. 53: 151-155.

Lipkin, E., Tal-Stein, R., Friedman, A. and Soller, M. (2008). Effect of quantitative trait loci for milk protein percentage on milk protein yield and milk yield in Israeli Holstein dairy cattle. Journal of Dairy Science. 91: 1614-1627.

Malarmathi, M., Senthil Kumar, T.M.A., Parthiban, M., Muthuramalingam, T. and Palanisammi, A. (2014). Analysis of beta-casein gene for A1 and A2 genotype using allele specific PCR in Kangayam and Holstein-Friesian crossbred cattle in Tamil Nadu. Indian Journal of Veterinary and Animal Sciences Research. 43: 310-315.

McLachlan, C.N.S. (2001). Beta-casein A1, ischemic heart disease mortality and other illnesses. Medical Hypotheses. 56: 262-272.

Mir, S.N., Ullah, O. and Shoikh, R. (2014). Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. African Journal of Biotechnology. 13: 555-565.

Mishra, B.P., Manishi, M., Prakash, B., Sodhi, M., Kapila, R., Kishore, A., Kataria, R.R., Joshi, B.K., Bhasin, V, Rasool, T.J. and Bujarbaruah, K.M. (2009). Status of milk protein, β-casein variants among Indian milch animals. Indian Journal of Animal Sciences. 79: 722-725.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proceedings of the National Academyof Sciences of the United States of America. 70: 3321-3323.

Olenski, K., Kaminski, S., Szyda, J. and Cieslinska, A. (2010). Polymorphism of the beta-casein gene and its associations with breeding value for production traits of Holstein-Friesian bulls. Livestock Science. 131: 137-140.

Rajendran, R., Raja, T.V., Thiruvenkadan, A.K., Nainar, A.M. and Thangaraju, P. (2008). Morphobiometrical characteristics and management of Umblachery cattle from coastal region of Tamil Nadu, India. Livestock Research for Rural Development. Volume 20, Article #40. Retrieved April 19, 2020, from http://www.lrrd.org/lrrd20/3/raje20040.htm.

Ramesha, K.P., Rao, A., Basavaraju, M., Alex, R., Kataktalware, M.A., Jeyakumar, S., Varalakshmi, S. (2016). Genetic variants of β-casein in cattle and buffalo breeding bulls in Karnataka state of India. Indian Journal of Biotechnology. 15: 178-181.

Sharma, V., Sharma, N., Jawed, B., Nautiyal, S.C. and Singh, R.K. (2013). High resolution meltcurve analysis for the detection of A1, A2 β-casein variants in Indian cows. Journal of Microbiology and Biotechnology Research. 3: 144-148.

Sulimova, G., Azari, M.A., Rostamzadeh, J., Abadi, M.M. and Lazebny, O. (2007): κ-casein gene (CSN3) allelic polymorphism in Russian cattle breeds and its information value as a genetic marker. Russian Journal of Genetics. 43: 73-79.

Sun, Z., Zhang, Z., Wang, X., Cade, R., Elmer, Z. and Fregly, M. (2003). Relation of beta-casomorphin to apnea in sudden infant death syndrome. Peptides. 24: 937-943.

Tailford, K.A., Berry, C.L., Thomas, A.C. and Campbell, J.H. (2003). A casein variant in cow's milk is atherogenic. Atherosclerosis. 170: 13-19.

Thorsdottir, I., Birgisdottir, B.E., Johannsdottir, I.M., Harris, D.P., Hill, J., Steingrimsdottir, L. and Thorsson, A.V. (2000). Different (beta-casein) fractions in Icelandic versus Scandinavian cow's milk may influence diabetogenicity of cow's milk in infancy and explain low incidence of insulin-dependent diabetes mellitus in Iceland. Pediatrics. 106: 719-724.

Threadgill, D.W., Womack, J.E. (1990). Genomic analysis of the major bovine milk protein genes. Nucleic Acids Research. 18: 6935-6942.