# Distribution of allelic and genotyping frequency of A1/A2 allele of beta casein in Badri cattle

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## ABSTRACT

Beta caseins (β-casein), the major milk protein, has two most important variants A1 and A2. A1 milk is considered as potential contributor to some of the important human diseases like diabetes and cardiovascular diseases. The present study was undertaken to explore the existing β-casein A1/A2 polymorphism in Badri cattle from Uttarakhand state of India using PCR-RFLP approach. A high frequency of A2 allele (0.88) was observed in Badri cattle with genotypic frequency for A2A2 and A1A2 to be 0.76 and 0.24 respectively. The analysis indicates that the Badri cattle is a good resource for A2 milk like other indigenous cattle breeds of India.

**Keywords:** Badri cattle, Beta casein, A1/A2 allele

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## INTRODUCTION

Milk is regarded as natural source of proteins, minerals and vitamins. Cow milk proteins are mainly of two types; caseins and whey proteins with caseins contributing about 80%. (Marten *et a1*.1994). The bovine casein gene cluster is located on BTA6 and comprises four casein genes, alpha S1, beta, alpha S2, and kappa casein, as well as five physically linked genes (Rijnkels 2002). It is important to study single nucleotide polymorphism (SNP) located in the coding region of genes as these variations lead to differences in quality or quantity of milk. Amongst the caseins, beta casein (β-casein) ranks as second most abundant protein in bovine milk (40%). β-casein is a source of active peptides, mainly opioids (Bell *et al.* 2006; Givens *et al.* 2013; Nguyena *et al.* 2015). These bioactive peptides released during *in vivo* or *in vitro* digestion of milk differ according to the polymorphism in beta casein. Some of these might be associated with health hazards and hence it is important to study the prevalence of different allelic variants of beta casein in our precious native cattle breeds.

The bovine β-casein gene spans a region of 8.5 kb on chromosome 6 with 9 exons and 8 introns. During the course of evolution, different mutations have led to generation of 12 genetic variants of beta casein *viz.,* A1, A2, A3, B, C, D, E, F, H1, H2, I, and G have been reported (Farell *et al.* 2004). Amongst these variant A1 and A2 are the most common, B is less common and A3 and C are rare (Farrel *et al.,* 2004). The A1 and A2 variants differ at amino acid position 67 of the beta casein gene: A2 (CCT, proline) A1 (CAT, histidine). This polymorphism leads to key conformational changes in the secondary structure of expressed β-casein protein (Eliott 1999, McLachlan 2001) and different bioactive peptides are generated on digestion of specific variants. Gastrointestinal proteolytic digestion of A1 β-casein releases a 7 amino acid bioactive peptide 'opioid' called beta-casomorphins-7 (BCM 7) in small intestine, while proline in A2 milk at 67 position prevents the split at this site and generates 9 amino acid peptide (Hartwig *et al.* 1997, Elliot *et a1.* 1999) called beta-casomorphins-9 (BCM9). BCM7 binds to the mu-opioid receptors distributed in the central nervous system, gastrointestinal tract and interfere with different pathways. BCM-7 is considered to be a risk factor for typeI diabetes (DM-I), coronary heart disease (CHD), arteriosclerosis, and sudden infant

death syndrome (Langersen and Elliot 2003, Truswell 2005; Kamiński *et al.,* 2006; Caroli et al., 2009; Pal *et al.,* 2015; Sodhi et al., 2012).

A number of studies have been carried out across the globe and breeds to delineate the frequency of A1/A2 allele of β-casein. In such studies, a high frequency of A1 allele was observed in taurine breeds (Holstein Friesian, Ayrshire and British Shorthorn) of western countries (EFSA, 2009; Kaminski *et al.* 2007) whereas frequency of A2 allele was high in Indian native cattle breeds (Mishra *et al*.2009; Sodhi *et al.* 2012) as well as in African cattle of indicine origin. The role of A1 beta casein as undesirable variant has led to the need for genotyping cows for A1/A2 allele of beta casein and making selection based on their polymorphism.

Badri is the only registered cattle breed of Uttarakhand. It is found in the hill districts of Uttarakhand and is the main source of milk for human population residing there. With estimated population of around 16 lakhs (ICAR, 2014) Badri cows contribute to 76.29 % of total cattle population of the state. In the breeding tract, the owners allow the animals to graze in the fields that adds to the medicinal values to its products (Banga et al. 2005). The state of Uttarakhand has been declared as organic farming state and Badri cattle with very high potential for producing organic food is considered as a gifted animal to the people of Uttarakhand. It plays a very significant role in development of Uttarakhand as a real organic state. Keeping in view the importance of Badri cattle to the local people, the present study was undertaken with the objective to delineate the frequency of A1/A2 allele of β-casein variations in this important breed

## MATERIALS AND METHODS

A total of 90 blood samples of Badri cattle were analysed for assessment of allelic and genotypic frequency of A1/A2 alelle of beta casein. The blood samples from ninety unrelated, true to the breed animals of Badri cattle were collected from its breeding tract. Badri cattle is mainly distributed in two major regions, Garhwal and Kumaon of Uttarakhand. Fourty two blood samples were collected from different villages of Bageshwar, Almora, Champawat and Nanital districts of Kumaon region. In Gharwal regions, different villages of Uttarkashi, Rudraprayag, Chamoli and Tehri Gharwal were covered to collect 48 samples. The animals were genotyped at the beta-casein locus by PCR-RFLP method using he methodology by (Lien *et al.* 1992).The Genomic` DNA was isolated using the standard protocol of SDS-proteinase K digestion followed by phenol: chloroform extraction (Sambrook *et al.* 1989). The primer pair CASB 122 L ( 5 ´ - G A G T C G A C T G C A G A T T T T C A A A TCAGTGAGAGTCAG-3´) and CASB67 R Forward (5´-CCTGCAGAATTCTAGTCTATCCCTTCCCTGGGCCCATC G-3´) were used to amplify the 251 bp fragment of exon 7 of the β-casein gene.

PCR was performed in a reaction volume of 25u1 with 150-200ng of genomic DNA, 1x reaction buffer, 1 unit of Taq DNA Polymerase, 1.5 mM MgC12, 200 µm each dNTPs and 5 pmol of each primer. To carry out the PCR reaction the thermocycler machine was programmed for specific conditions: an initial denaturation at 95C for 2.30 min., followed by 30 cycles of 94 C for 60 sec., annealing temperature 63$^0$C for 60 sec., and 72C for 60 sec with a final extension for 10 min. at 72C. After the completion of PCR reaction the amplification was checked by electrophoresis on 1.5% agarose gels stained with ethidium bromide. The amplified PCR products were digested with one units of *Taq-I* restriction enzyme at 65 °C for 3-4 hours followed by 20 min. of inactivation at 85C. The digested products were resolved on 3.5% agarose gel in 1X TAE buffer and genotypes were recorded according to fragment size.

## RESULTS AND DISCUSSION

To assess the allelic and genotypic frequency of

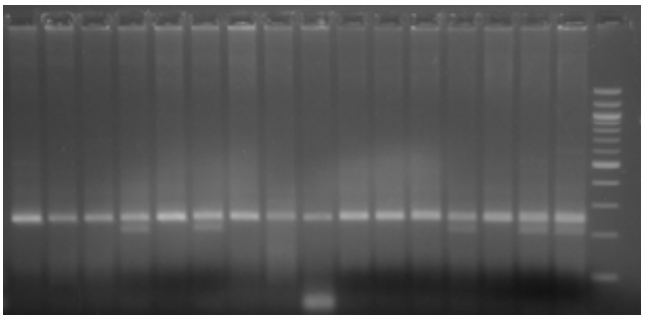

***Figure 1:*** *PCR-RFLP of β-casein/TaqI showing A2A2 and A1A2 genotypes in Badri cattle.*

**Table1:** Allelic and genotypic frequencies of A1/A2 variant of β-casein gene in Badri cattle

| Region of sample collection | n | Allelic frequency | | Genotypic frequency | | |
|---|---|---|---|---|---|---|
| | | A2 | A1 | A2A2 | A1A2 | AlAl |
| Garhwal | 42 | 0.88 | 0.12 | 0.76 | 0.24 | 0 |
| Kumaon | 48 | 0.87 | 0.13 | 0.75 | 0.25 | 0 |
| Average | 90 | 0.88 | 0.12 | 0.76 | 0.24 | 0 |
| *Average* | *90* | *0.88* | *0.12* | *0.76* | *0.24* | *0* |

A1/A2 allele of beta casein in Badri cattle, 251bp region of exon 7 of beta casein gene harboring A1/A2 variants was amplified. The restriction digestion of PCR amplicon with Taq1 resulted in 3 different fragment combination: only one fragment of 251bp for A2A2 genotype, two fragments of 213bp and 38 bp for A1A1 and 251 bp, 213 bp and 38 bp fragments for A1A2 genotype (Figure 1).

The allelic frequency distribution data indicated predominance of A2 allele (0.88) in Badri cattle. A1 allele was observed only in few animals with a frequency of 0.12. The observed genotypic frequency for homozygous A2A2 and heterozygous A1A2 genotype were 0.76 and 0.24, respectively (Table 1). None of the animal showed homozygous A1A1 genotype. There was no significant difference in the allelic and genotypic frequencies between the Badri animals collected from Garwal and Kumaon region (Table1). Badri animals from Garwal region showed slightly higher frequency of A2 allele and A2A2 genotype.

Overall, the data clearly indicates that with higher frequency of A2 allele, Badri cattle is a good resource of A2 milk. Likewise other Indian cattle breeds none of animal was homozygous for A1A1 genotype (Table 2). However, the frequency of A2 allele (0.88) in Badri cattle is slightly on lower side than the average frequency of A2 allele across the other Indian native cattle breeds (0.987).

Similarly the frequency of A2A2 genotype was also on lower side (0.76) in comparison to other Indian native cattle breeds 0.97 (Table 2). Conversely, frequency of A1 allele (0.12) and heterozygous A1A2 genotype (0.24) was on higher side for Badri in comparison to other Indian cattle breeds (0.013 and 0.026 for A1 allele and A1A2 genotype respectively; Table 2). The gene/genotypic frequencies of beta casein variants for Badri cattle were close to that of

**Table 2:** Allelic and genotypic frequencies of A1/A2 variant of β-casein gene in Indian native cattle breeds

| Cattle breed | N | Allelic frequency | | Genotype frequency | | |
|---|---|---|---|---|---|---|
| | | A1 | A2 | AlAl | A1A2 | A2A2 |
| Amritmahal | 37 | 0 | 1 | 0 | 0 | 1 |
| Gir | 45 | 0 | 1 | 0 | 0 | 1 |
| Hariana | 48 | 0 | 1 | 0 | 0 | 1 |
| Kangayam | 48 | | | | | |
| | 0 | 1 | 0 | 0 | 1 | |
| Kankrej | 32 | 0 | 1 | 0 | 0 | 1 |
| Kherigarh | 23 | 0.109 | 0.891 | 0 | 0.218 | 0.783 |
| MalnadGidda | 47 | 0.096 | 0.904 | 0 | 0.191 | 0.809 |
| Malvi | 44 | 0 | 1 | 0 | 0 | 1 |
| Mewati | 40 | 0 | 1 | 0 | 0 | 1 |
| Nimari | 45 | 0 | 1 | 0 | 0 | 1 |
| Rathi | 46 | 0 | 1 | 0 | 0 | 1 |
| Red Kandhari | 39 | 0 | 1 | 0 | 0 | 1 |
| Red Sindhi | 33 | 0 | 1 | 0 | 0 | 1 |
| Sahiwal | 47 | 0 | 1 | 0 | 0 | 1 |
| Tharparkar | 44 | 0 | 1 | 0 | 0 | 1 |
| Mean | 618 | 0.013 | 0.987 | 0 | 0.026 | 0.974 |

*(Adapted from Mishra et al. 2009)*

MalnadGidda and Kherigarh (Table 2) wherein lower frequency for A2 allele and A2A2 genotype and higher frequency for A1 allele and A1A2 genotype was observed as compared to rest of the Indian breeds analyzed. Comparatively higher frequency of A1 allele and A1A2 genotype in Badri cattle as compared to other Indian cattle breeds might be attributed to the crossbreeding of local Badri cattle with Jersey cattle in recent years for higher milk production, though care was taken to collect samples of only true to the breed animals based on morphological characters. Taken together, the present study highlights the predominance of A2 allele in Badri cattle. As A2 milk is considered safe for human health, the outcome adds value to this native breed of Uttarakhand that in turn will help in its better utilization and long-term conservation.

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