Milk proteins, health issues and its implications on National Livestock Breeding Policy of India

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Increasing evidence supporting the correlation between bovine beta-casein and disorders in milk consumers has led to the development and popularity of A2 milk and milk products worldwide. The indigenous cattle population of India harbours the preferred A2 allele of beta casein; however, genetic improvement of this cattle population by crossbreeding with exotic breeds is spoiling the gene pool as most of the exotic breeds contain A1 allele of the gene. In this study, we aim at evaluating the status of beta-casein in the Indian dairy sector and provide a discussion of future implications to the A2 milk industry. Our results show that the genotypic frequencies of A1 and A2 genes in a sample cattle population were 0.365 and 0.635 respectively. Based on the pure nature of the A2 allele in indigenous cattle, the presence of the A1 allele was assumed to be attributable to crossbreeding with exotic cattle breeds. Current options to drift the frequency of herds towards A2 are highly limited, raising serious questions regarding breeding policies in India and the lack of availability of certified A2 semen straws. The absence of any official standards and certification procedures in the country for A2 milk and milk products aggravates the situation from a food safety perspective. The future focus of sorted sexed semen and embryo transfers by the Department of Animal Husbandry, Dairying and Fisheries in India for dairy development should include A2 certification as an integral component. The Food Safety and Standards Authority of India should define and establish standard protocols for certification of A2 milk and milk products to safeguard the rights of consumers against the potential food fraud.

Keywords: Beta-caseins, genotyping, livestock breeding policy, milk safety, milk proteins.

BOVINE milk plays a vital role in human diet and has many beneficial effects, including anticarcinogenic, immunomodulatory, antimicrobial, antihypertensive and hypocholesterolemic effects[1]. Proteins are important constituents of milk and account for approximately 3.5% of milk components[2]. Milk proteins can be categorized into two major groups, i.e. casein and whey proteins, which account for 80% and 20% of milk proteins respectively[3]. These milk proteins are products of the transcription and translation of host genes. The genes *CSN1S1*, *CSN1S2*, *CSN2*, and *CSN3* are located on chromosome 6 of the bovine genome encode casein alpha s1, alpha s2, beta, and kappa respectively[4]. Any differences in DNA sequence due to deletions, insertions, or exchanges of one or more bases can result in a different protein variant. Beta-casein constitutes up to 45% of total casein and exhibits 12 genetic variants (A1, A2, A3, B, C, D, E, F, G, H1, H2 and I). The variants A1 and A2 are most common worldwide, followed by variant B; the remaining variants are rare[5].

Differences in protein components can influence the effects of milk on human health, for example, modulating hypo-allergenic reactions to particular proteins, such as αs1-casein, αs2-casein, and β-lactoglobulin, and altering the effects of metabolites formed by proteolysis during processing or gastrointestinal digestion[6]. The gastrointestinal digestion of beta-casein (variant A1) leads to the formation of beta-casomorphin 7 (BCM7), which is an important bioactive peptide with strong opioid activity (Figure 1). This process can be attributed to the presence of histidine at position 67, which readily allows the cleavage of amino acid chains (Figure 1). However, the proline (variant A2) at this position hinders this cleavage[7]. Thus, after consumption of A1 milk, A1 beta-casein releases large amounts of beta-casomorphin7 (BCM7) in the intestine, whereas A2 milk does not[8]. BCM7 has been linked to increased risk of human diseases, such as type I diabetes mellitus[9,10], coronary heart disease[11], arteriosclerosis[12], sudden infant death syndrome[13], schizophrenia and autism (Figure 1)[14].

A1 beta casein is an undesirable variant due to its negative health implications; and this has led to the development and marketing of premium A2 milk and milk products in New Zealand[15]. This concept has also spread to different countries, including India. Thus, various milk products are marketed in India as having pure A2 casein (e.g. Vita A2 milk, Deshi A2 Amul). Only animals with genotype A2A2 can produce pure A2 milk. A1 beta-casein is absent in milk from pure Indian zebu cattle[7], suggesting an outstanding opportunity for the Indian dairy industry to grow both nationally and internationally. However, this issue is far more complex involving problems of certification, the unorganized nature of the dairy industry, consumer fraud and food safety standards.

In this study, we used the Andaman and Nicobar Islands for a case study owing to the geographical isolation, limited free movement of animals to facilitate tracking and traceability, high demand for establishment of a dairy sector, and the ability to study the potential effects of interventions in future without any significant variables. There are no comprehensive genetic data available on A1/A2 beta-casein variants in cattle in the Andaman and Nicobar Islands. This is the first study to attempt to determine the frequency of A1/A2 beta-casein

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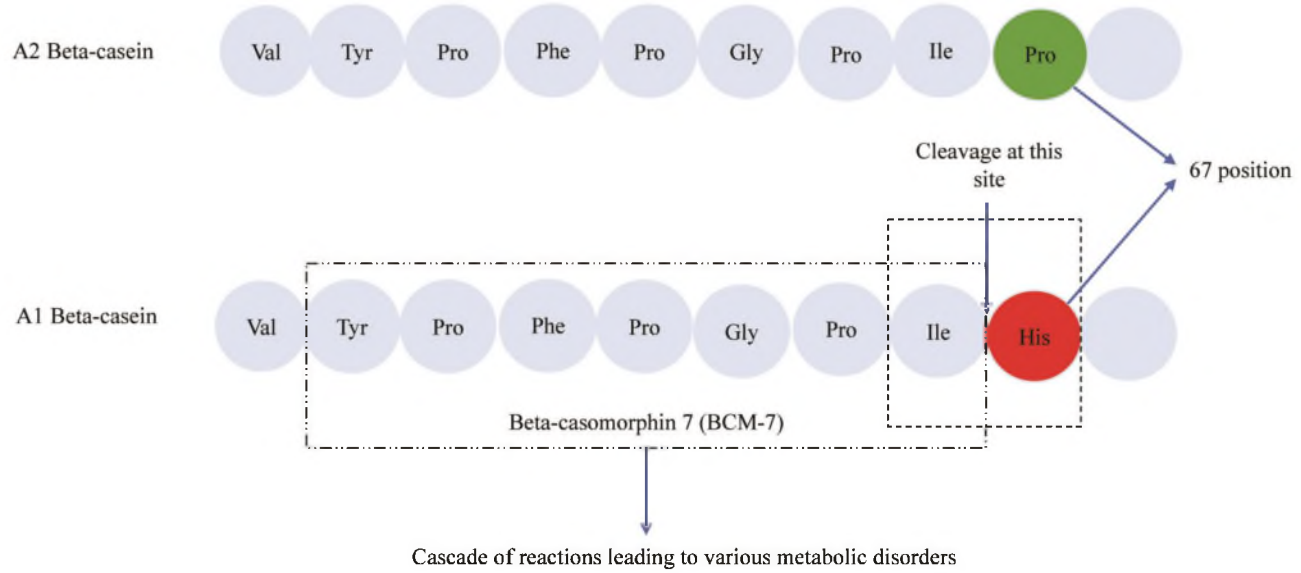

Figure 1. Differences between A1 and A2 beta-casein.

variants in animals from these islands, and thereby evaluate the quality of milk produced by these animals. Based on laboratory findings, a set of interventions are proposed to yield certified premium A2 milk, achieve better returns for farmers, and improve consumer protection. We expect that this study may have implications with regard to future breeding policies and food safety standards in India.

In the present study, blood samples were collected from the Cattle Holding Farm, Livestock Farm Complex, Dollygunj, Andaman and Nicobar Islands. This farm is a central dairy training-cum-demonstration unit for farmers in the region. In addition to the training programmes, this farm is also involved in the distribution of calves and heifers to progressive farmers. This farm is dependent on artificial insemination technology to breed cows. A total of 26 blood samples were collected from the farm and processed at the Division of Animal Science, ICAR-Central Island Agricultural Research Institute, Port Blair.

Blood samples (5 ml/animal) were collected from the jugular vein in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes.

The samples were transported immediately to the laboratory under cold storage condition using ice packs. Genomic DNA was extracted by enzymatic digestion using proteinase K followed by routine phenol–chloroform extraction methods[16]. The primers and restriction fragment length polymorphism polymerase chain reaction (PCR) protocol employed to amplify the 251-bp fragment of exon 7 of the β-casein gene and distinguish the A1 and A2 variants that were similar to those reported by Lien *et al.*[17].

PCR was performed using 150–200 ng of genomic DNA in a reaction volume of 25 µl containing 5 pmol of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$ and 1 U *Taq* DNA polymerase. PCR was carried out in a thermal cycler using the following cycling programme: 95°C for 5 min, followed by 30 cycles at 94°C for 30 sec, 63°C for 40 sec and 72°C for 20 sec, with a final extension at 72°C for 3 min.

The purified PCR products were digested with 5 U *Taq* I restriction enzyme at 65°C for 3 h. The digested products were resolved on 3.5% agarose gels in 1× TAE buffer, and genotypes were recorded according to the fragment size. Based on genotyping results, analyses were carried out with regard to the development of a replacement herd to yield pure A2 milk. An overview of the methodology used for genotyping of cattle for the A1/A2 allele is presented in Figure 2.

The detailed genotypes along with the phenotypic characteristics in the examined population are presented in Table 1. In our sample population, the most common allelic variant of beta casein was A2, followed by A1, with frequencies of 0.635 and 0.365 respectively. The heterozygous A1A2 genotype was the most frequent (frequency: 0.73), followed by the A2A2 genotype (frequency: 0.27) (Figure 3). No animals were found with the homozygous A1A1 genotype (Table 2). The phenotypic characteristics were not true representatives of genotype of the animals for A1/A2 allele, particularly in crossbred populations, as the degree of purity of the animals could not be established (e.g. animal tag nos. 63, 67 and 72). Hence, the screening of individual animals is necessary to make a valid claim regarding the genotype of the cattle and the potential for obtaining high-quality milk in terms of beta-casein. Thus, our findings suggest that the claims made by products available on the Indian market are questionable.

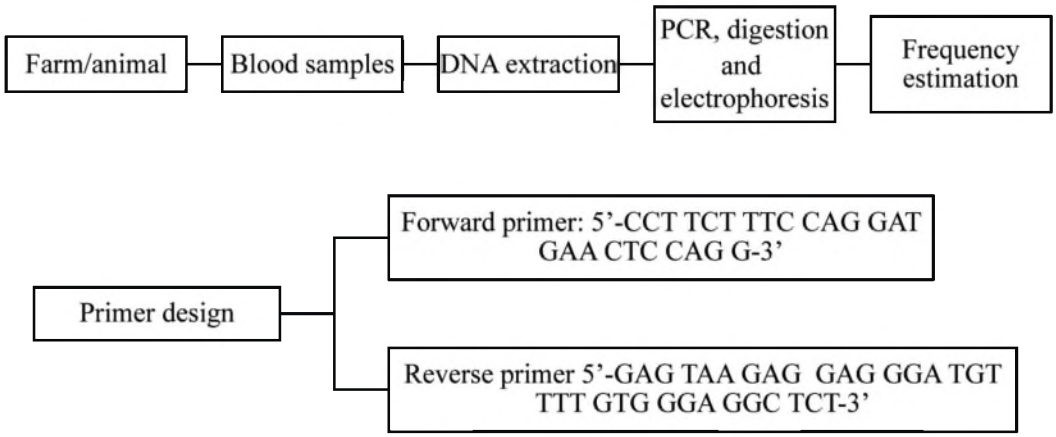

Figure 2. Methodology for genotyping of cattle for the A1/A2 allele from blood samples.

Table 1. Phenotypic characteristics of animals and their genotypes in the sample population

	Description				
Tag no.	Phenotypic breed characteristics	Age group	Physiological stage	Sample ID	Result
001	Jersey cross	Heifer	AI done	30 AD	A1A2
002	Jersey cross	Heifer	Pregnant	26Z	A1A2
004	HF cross	Heifer	Pregnant	29AC	A2A2
007	Jersey cross	Heifer	–	28AB	A1A2
009	HF cross	Heifer	–	27AA	A1A2
051	Jersey cross	Adult	Milking	9I	A1A2
052	Jersey cross	Adult	Milking	12L	A2A2
057	Jersey cross	Adult	Milking	21U	A1A2
060	Jersey cross	Adult	Milking	14N	A1A2
062	HF cross	Adult	Milking	15O	A1A2
063	Non descriptive	Adult	Milking	8H	A2A2
064	Jersey cross	Adult	Milking	18R	A1A2
065	HF cross	Adult	Milking	11K	A1A2
066	HF cross	Adult	Milking	20T	A1A2
067	Non descriptive	Adult	Milking	16P	A1A2
069	Jersey cross	Adult	Milking	17Q	A1A2
070	Jersey cross	Adult	Milking	7G	A1A2
072	Not descriptive	Adult	Milking	2B	A1A2
073	HF cross	Adult	Milking	6F	A1A2
076/0175	Jersey cross	Adult	Milking	4D	A2A2
095	Jersey cross	Adult	Milking	13M	A1A2
096	Jersey cross	Adult	Milking	10J	A1A2
0194	HF cross	Adult	Milking	5E	A2A2
0217	Jersey cross	Adult	Dry: Pregnant	24X	A2A2
0237	Jersey cross	Adult	Dry: Pregnant	25Y	A1A2
0299	Jersey cross	Adult	Milking	19S	A2A2

The prevalence of A1 alleles in the cattle in India can be attributed to crossbreeding programmes used in dairy development to upgrade the production potential of the animals. The Department of Dairying, Animal Husbandry, and Fisheries under the Ministry of Agriculture (GoI) is the primary agency focusing on issues of breeding policy and dairy development at the national level. This department focuses on artificial insemination (AI) of the animals to achieve genetic progress. The National Programme on Bovine Breeding and Dairy Development focuses on the development of the field AI network through the Multipurpose AI Technician in Rural India programme. Its target is to produce 100 million semen doses annually and increase the breedable population under AI from 24% to 35%. To meet the demand for exotic semen in the country, it may also be necessary to import 400 bulls/equivalent embryos of HF and Jersey breeds[18].

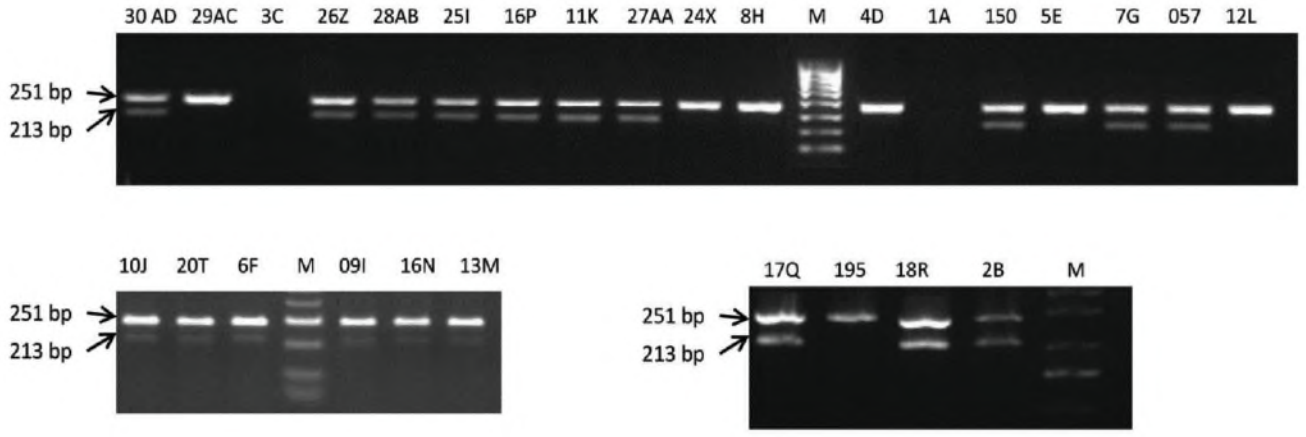

Figure 3. Genotypes of beta-casein in the samples (3.5% agarose gels). M: 100 bp DNA ladder.

Table 2. Priority ranking for the development of a pure A2 replacement herd

Sample population	26 crossbred animals			Location: LFC Dollygunj, Port Blair
Genotype	A1A1	A1A2	A2A2	Pure certified A2 milk
Number of animals	0	19	7	Animals with tag no. 4, 52, 63, 76,
Genotype frequency	0.00 (0/26)	0.73(19/26)	0.27(6/26)	194, 217 and 299

Scenario	Requirement	Probability of female A2A2 progeny			Remarks
Present scenario	No certified A2 semen straws	NP	?	?	High risk of contamination of even the pure existing genotype
1	Certified A2 semen straws	NP	25%	50%	Need of the hour
2	Certified A2 sorted semen straws	NP	50%	100%	Future path breaking objective
3	Certified A2A2 embryos	50%	50%	50%	Not favourable
4	Certified A2A2 female embryos	100%	100%	100%	Ideal scenario for A1A2 animals
Ideal scenario	(2) for A2A2 animals + (4) for A1A2 animals	100%	100%	100%	100% pure replacement stock with less cost

NP, Not possible. ?, Signifies the unpredictable nature of output.

The presence of large crossbred cattle populations and higher proportions of the A1 allele in exotic animals necessitates careful screening of animals and fine-tuning of breeding programmes to drift herds towards the A2 genotype. Such an approach would be an effective measure to prevent the dissemination of the undesirable A1 allele in our existing A2-predominant indigenous cattle populations. However, information regarding the screening of bulls for the A1A2 genotype in government semen stations is not available, and there are no certified A2 semen straws available in the India market. Thus, there is a major risk associated with contamination of the genetic potential of the indigenous cattle population, which is considered pure for A2 allele.

Thus, in the existing scenario, few options are available for transformation of existing herds to produce pure certified A2 milk. Additionally, most breeding programmes in the country depend on AI in cattle. Therefore, screening of all semen stations in the country for genotyping of their bulls to produce certified A2 semen straws is necessary.

Available technology for AI will have limited impact on animals with the A1A1 or A1A2 genotype because the probabilities of achieving homozygous female A2 offspring are only 0% and 25% respectively (Figure 4 and Table 2). Thus, the chance that the replacement herd will be able to produce the desired quality of milk after the generation gap of 4 years is low. Thus, other alternatives, such as embryo transfer technology, are required. In a single generation, the replacement herd can be converted to produce pure A2 milk, provided that the genotype of the parent stock producing embryos and semen has been verified. The priority-based ranking of the alternatives corresponding to the parental genotype is shown in Table 2.

If claims of the disorders linked to the consumption of A1 milk are true, then this type of milk may be a potential biological hazard for consumers. Accordingly, it may be necessary to monitor the status of A1/A2 alleles in our dairy animals and evaluate the products as a cautionary measure to benefit consumer health. With increasing awareness of health and food quality, A2 milk is gaining

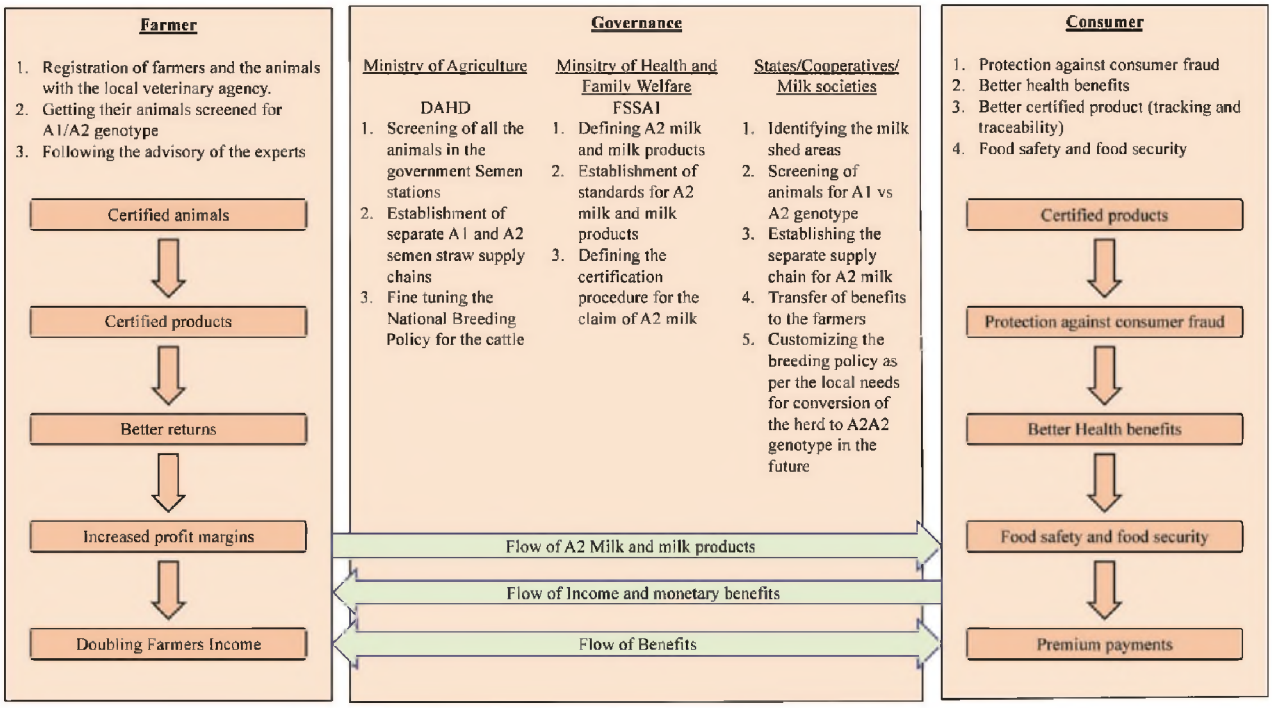

Figure 4. Good governance, doubling farmers' income and food safety: A2 milk in India.

popularity, and its demand is on the rise. The Indian market has a range of milk and milk products claiming to be of A2 type. However, FSSAI has not established a definition, standards, or certifying procedure for such products. Additionally, the options available to consumers with regard to food fraud related to this issue are limited. As 68.4% of the milk in India does not meet the basic quality parameters[19], Indian consumers are at potential risk of violation of food safety rights.

The development of supply chains for pure A2 milk requires the certification and traceability of the origin of the milk and animal. In New Zealand, a DNA-based kit has been developed to screen cattle herds, and A2 milk is marketed as a premium brand (A2 Corporation, 2006). Similar trends can be seen in the Indian market with leading brands such as Amul and Vita (Haryana), which have launched premium A2 milk and milk products. The premium payments associated with A2 milk can motivate farmers and result in better returns provided the benefits are transferred to the ultimate producer.

$$\text{No. of days for recovery of screening cost} = \frac{\text{Cost of screening}}{\text{Milk production of the animals} \times \text{premium payment}}.$$

At present, the cost of screening a sample for the A1A2 genotype in ICAR-National Bureau of Animal Genetic Resources, Karnal is Rs 800/sample[20]. If the benefits of premium payments are transferred to the farmer, e.g. Rs 10, and the milk production is 10 litre/day, then the testing cost is recovered in eight days, and the return then contributes to the profit margin of farmers and is of direct benefit to the farmers' income. This can be an important consideration for the Indian government's flagship programme called 'doubling farmer's income'.

Issues with A2 milk and milk products are gaining importance both at the national and international levels. However, no significant changes to legislation or regulations have been made in India. There are still lack of standards and certification procedures for A2 milk products, and clear-cut policies are required to address this in an organized manner and to transfer the benefits to livestock farmers and consumers. The interventions required by different government agencies and their associations with desirable outcomes are given in Figure 4.

In summary, the first step is recognition of the issue as a potential biological health hazard by the Departments of Food Safety, Health, and Animal Husbandry or establishment of a separate 'National One Health Agency'. The existing scenario demands the immediate attention of all stakeholders to address the serious issues regarding A2 milk according to food safety principles and prevent further spoilage of the indigenous gene pool of the cattle. The production and availability of certified A2 semen is the need of the hour. Further the Department of Animal Husbandry, Dairy and Fisheries should focus on sexed semen and embryo transfer technologies. The Food Safety Standards Authority of India also must define and establish standard protocols for claims of A2 milk and

milk products by producers and processors to safeguard the rights of the consumer against the potential food fraud.

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