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Analyses of A1/A2 type-casein variations in Tharparkar and Kankrej breeds of cattle

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Abstract
The benefits of milk as a food source for nutrients have long been recognized. Most milk proteins (80% and 10%, respectively) are made of casein and whey. One of the essential milk proteins in cows, -casein (-CN), is encoded by a collection of highly polymorphic genes, leading to the production of 12 distinct protein variants. A1 and A2 are among their most prevalent variations. Comparing *Bos taurus* and Bos indicus, the latter has a larger percentage of the A2 type -CN. The synthesis of -casomorphin-7 (BCM-7) during gastrointestinal proteolytic digestion is a risk for milk carrying A1 or A1/A2 -CN variations, which has been connected to a number of health problems in humans. The purpose of the present study was to examine the incidence of -CN mutations among Tharparkar and Kankrej.

Keywords: A1/A2, casein, prevalence, bovine

Introduction
Milk is frequently viewed as a wholesome food and essential part of the diet. The primary producers of milk worldwide are cows, buffaloes, goats, sheep, and camels, which account for 85%, 11%, 2%, 1.4%, and 0.2% of all milk production, respectively [1]. With a global herd capacity of 264 million cows, cows are the main source of milk production, contributing 600 million tonnes (83% of all milk produced) of milk annually [1]. With 178 million cattle, India produces the most milk globally—roughly 182.16 million tonnes [2]. The average amount of milk per person available globally in 2014–2015 was 322 g/day [3]. India consumes more milk than the world average when compared to emerging nations, highlighting the need to pay attention to the advantages of drinking milk to health.

Milk proteins are mostly made up of casein and whey proteins (80% and 10%, respectively). One of the main milk proteins in cattle is -casein (-CN). Like all other milk proteins, -CN is a highly polymorphic protein. There are 49 different milk protein variants that Farell *et al*. discovered in 2004. 12 genetic variants (A1, A2, A3, B, C, D, E, F, H1, H2, I, and G) have so far been linked to –CN [4]. The most common variants in dairy cattle breeds are A1 and A2 -CN, whereas B and A3 -CN are less common [5]. βAccording to their propensity to emit -casomorphin-7, -CN variants can be classified into two groups, A1 type and A2 type (Fig. 1). Variants of B, C, F, G, and D are included in the first group, whereas A3, E, D, H1, H2, and I [6] are included in the second. The codon CAT (proline) was altered to CCT (histidine), resulting in the variants A2 and A1. It is susceptible to gastrointestinal proteolytic digestion for the release of -casomorphin-7 (BCM-7), which has been linked to a number of human health issues, including SIDS (Sudden Infant Death Syndrome), schizophrenia, autism, milk allergies [7], and arteriosclerosis [8]. This is due to the presence of histidine at position A1 as opposed to proline at position A2. Epidemiological, *in vivo*, and *in vitro* studies have linked A1/A2 milk consumption to both positive [9, 10] and adverse [11] health problems.

This mutation also has evolutionary significance since Bos indicus exhibits the A2 primitive type more frequently than *Bos taurus*. The majority of taurine breeds domesticated in America, Europe, and Australia are taurine, which have been selectively bred for higher milk production, resulting in a lower frequency of the A2 allele (with a few exceptions, such as the Guernsey and Fleckvieh breed). Exceptions include the Guernsey and Fleckvieh breed. The A2 gene is present at a higher frequency in Indian cattle because they have naturally evolved without being subjected to selection pressure. Numerous epidemiological studies in the European population have demonstrated a high association between consumption of A1 milk and type I diabetes [12, 13, 14], ischemic heart disease [12, 10], and neurological disorders [15].

As a result, the current study was conducted to determine the decrease in A2 allele frequency in native cattle (Tharparkar and Kankrej). The A2 gene pool will be kept in the local herd as a result, making it simpler to design breeding plans in the future.

Materials and Methods

Each of the 30 animals from the Tharparkar and kankrej breeds was chosen from the LRS (CVAS, Rajasthan University of Veterinary and Animal Sciences, Bikaner), LRS (Kodamdesar, Bikaner), (CVAS, Rajasthan University of Veterinary and Animal Sciences, Bikaner) facilities, in that order. Mastitis had been ruled out in every cow. A Qiagen QIAamp® DNA micro kit was used to extract the genomic DNA after the blood was taken from the jugular veins. The DNA was then taken out and put into a nanodrop to be checked for concentration and purity. Target ferments of -CN that had single-nucleotide polymorphisms (SNPs) were amplified in accordance with McLachlan's (2006) [13] A restriction site for a taqI enzyme is produced in the amplicon upon amplification thanks to the design of the primers CASB67 and CASB122. 200 ng of genomic DNA was used in the PCR along with 200 M of each dNTP, 1 U of Taq polymerase, and a final volume of 5 pmol of primers (GoTaq® PCR Core System I, Promega). Following are the PCR cycles: 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 62 °C for 45 sec, 72 °C for 30 sec, and a final extension of 7 min. For CASB67/122 (Fig. 1 and 2), the PCR was performed on a 3% agarose gel at 100 V for an hour to identify the product sizes of 251and 116 bp. The final product was digested in a 25-liter reaction that contained 15 liters of PCR product, 2.5 liters of 10-fold NEB CutsSmart buffer, 5 units of TaqI enzyme for the CASB67/122 primer product.

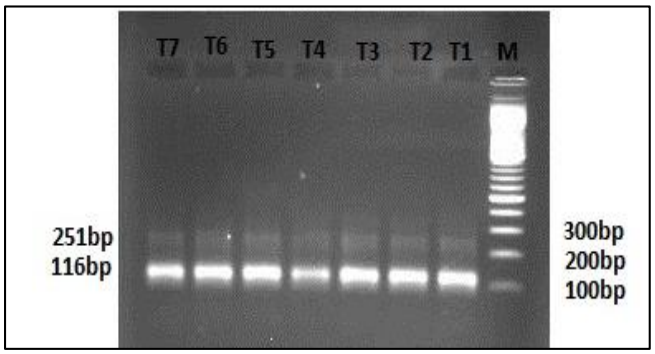

Fig 1: Tharparkar cattle's bovine -casein gene amplification results on a 3% agarose gel. Line M shows a 100 bp DNA marker, while the samples T_1, T_2, T_3, T_4, T_5, T_6, and T_7 have 251 bp and 116 bp bands, respectively.

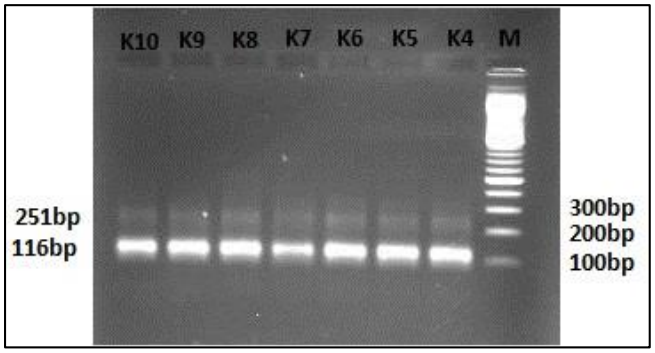

Fig 2: On a 3% agarose gel, the Bovine -casein gene of five Kankrej cattle was amplified. Line M shows a 100 bp DNA marker, while the numbers K4, K5, K6, K7, K8, K9, and K10 show samples with 251 bp and 116 bp bands, respectively.

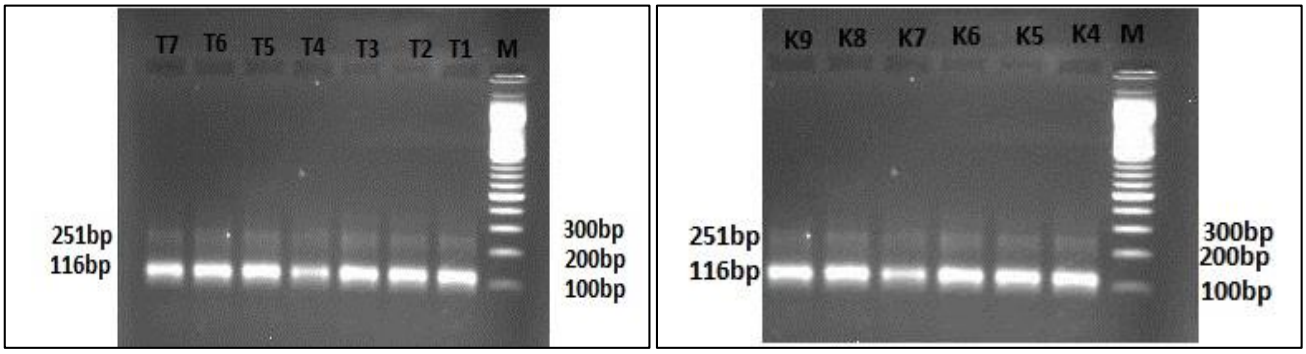

Fig 3: Seven Tharparkar cattle Five Kankrej cattle and were used in the amplification of the bovine -casein gene on a 3% agarose gel. Line M shows a 100 bp DNA marker, while the numbers T_1-T_7 (Tharparkar breeds) and K4-K10 (Kankrej breeds), show samples with 251 bp and 116 bp bands, respectively.

Results and Discussion

All blood samples collected from Tharparkar, and Kankrej were analyzed for genomic DNA, which was then amplified using the polymerase chain reaction (PCR). Five random DNA samples from each community were initially collected in order to optimize the PCR conditions, including the $MgCl_2$ concentration and annealing temperature. One sets of primers for the -casein gene were used in the reaction mixture

The set of primers (reported by Lien *et al*., 1992) [16] allowed for amplification of the genomic DNA at 65 °C with 34 cycles for -casein. Tharparkar and Kankrej's genomic DNA samples were utilized to amplify the targeted location of the -CN gene using the PCR-RFLP primers previously reported. The samples were digested using the Taq1 enzymes, as demonstrated and explained in the material techniques. Since the -casein gene's primers have been created in such a way that they amplify a certain gene region uses a restriction site that is either naturally occurring or artificially created for a variety of restriction enzymes, the refere fragments generated by utilizing appropriate primer sets were as follows: Casein primer set no.1 has 251 and 116 bp for Tharparkar, and Kankrej were displayed in Figs. 1 and 2. A2 allele frequency was observed in almost all breeds of -casein, This supported earlier findings by Mishra *et al*. (2009) [17] that only the A2 allele was present in the native Indian milch breeds Gir, Tharparkar, Rathi, Red Sindhi, Sahiwal, and dual purpose breeds Kankrej and Hariana.. The A2A2 genotype was indicated by single bands of 251 and 116 base pairs in all thirty tharparkar and kankrej animals were at 58 °C for annealing. The intended fragment size of the -casein gene

fragment were effectively amplified by PCR using the ideal primer concentration and cycling parameter. samples (Table 1 and Fig 3). The 30 Tharparkar cattle, however, all displayed one bands of 251 and 116 bps, indicating that they were A2A2 carriers (Table 1 and Fig 3).

Table 1: Cattle breeds Tharparkar and kankrej's genotypes and allele frequencies (-CN gene)

Breed of cattle	Population size	Genotype Frequency			Gene Frequency	
		A1A1	A1A2	A2A2	A1	A2
Tharparkar	30	0	0	1	0	1
Kankrej	30	0	0	1	0	1

However, the current study discovered that tharparkar and Kankrej cattle had an A2 allele frequency of 1 and an A1 allele frequency of 0 (by utilizing Hardy Weinberg's Equation). In order to confirm the A1 allele's presence or absence in Tharparkar and Kankrej cattle. The results show 'A' all at unlike taurine cattle, which have undergone intense selection for the milk production trait, Indian cattle have evolved spontaneously without selection pressure for the characteristic. The A1 allele has a higher breeding value for milk production, as is well-known [17]. The genotype of the next generation may also be much more affected by mating a single male with an imbalanced number of females than would be anticipated in accordance with Hardy Weinberg's principle due to the farm's low number of sires (n=3).

Conclusion

The -CN gene (A1/A2) and genotype frequency in regional cow breeds of Western India, such as the Tharparkar and Kankrej, were identified in the current study. All Tharparkar and Kankrej breed animals had A2A2 genotypes. These indigenous cattle (Tharparkar and Kankrej) exhibit A2 type beta casein, which has greater health benefits, as evidenced by the absence of A1A2 and A1A1 genotypes. Additionally, it could be used as a breeding technique to increase A2 milk output.

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