ISSN: 2456-2912
VET 2024; SP-9(3): 08-11

www.veterinarypaper.com
Received: 13-02-2024
Accepted: 16-03-2024

Selvaramesh AS
Assistant Professor and Head, Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) Tamil Nadu, India

Narmatha N
Dean, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS). Tamil Nadu, India

Corresponding Author:
Selvaramesh AS
Assistant Professor and Head, Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS) Tamil Nadu, India

Identification of B casein gene for A1 and A2 allelic variants in Pulikulam cattle of Tamil Nadu

Selvaramesh AS and Narmatha N

Abstract
Polymorphism of β casein gene in cattle focus attention to many researchers in animal breeding and dairy sector. Among the twelve β casein variants A1 and A2 are the most common and in zebu cattle the most abundant variant is A2. Research studies shows that A1 allelic variants involved with few health hazards in human being over A2. This study was conducted to find the occurrence of β casein variants in Pulikulam cattle of Tamil Nadu. DNA was isolated from 100 numbers of pulikulam cattle. Polymorphic site was amplified with allele specific primers through Polymerase Chain Reaction. Frequencies of A1 and A2 alleles were 0.090 and 0.910 respectively. The values of experimental heterozygosity (Heobs), theoretical heterozygosity (Heexp), and polymorphism information content (PIC) were 0.1400, 0.1646 and 0.1504 respectively. Results shows high frequency of A2 allele and animals with A2 allele may be used for breeding plans to maintain A2 alleles.

Keywords: Beta casein gene, A1 A2 allele, pulikulam cattle

1. Introduction
Milk is one of the most indispensable commodities for all the human beings. Milk contains several nutrients and unique nutritional make up due to various factors and mainly it is due to breed variation. In milk proteins β casein is the largest milk protein and it is having 13 different forms. But the most familiar forms of β casein under discussion now a days are A1 and A2 (Farrell *et al.*, 2004) [6]. At 67th amino acid position A1 contains Histidine and A2 contains Proline (Jaiswal *et al.*,. 2014) [9]. Past research studies suggest that due to this amino acid change, consumption of raw milk or as processed milk products such as cheese contains A1 allele may be harmful. Since A1 milk contains Histidine which contains a very weak bond and easily breakdown in the gut by enzymatic reaction and in turn the beta casein produces beta casomorphin -7 in human gut which may cause significant risks to human beings such as sudden infant death syndrome Sun *et al.*, (2003) [18] type I diabetes Elliott *et al*. (1999) [4] human ischemic heart diseases McLachlan (2001) [13], Laugesen and Elliott (2003) [12], and atherosclerosis Tailford *et al.*, (2003) [20]. More over consumption of A1 milk may responsible for inflammatory response, and delayed GI transit time in feeding trials in laboratory animals Barnett *et al.*(2014) [1] Ul Haq *et al.*, (2014) [21]. More bloating and abdominal pain Ho *et al*., (2014) [8]; Jianqin *et al.*, (2016) [10]. Hence more adverse effects due to A1 allele focus the attention of the researchers to screen our cattle population carrying A2A2 genotypes. In India, farmers are neglected to rear native cattle due to low milk yield when compared to cross bred population which gives more milk but the recent years research findings suggest that our zebu cattle carries more frequency of A2 allele which has less harmful effect compared to A1 allele. But for the conclusive evidence about the beneficial or hazardous effect of A2 or A1 allele is still not much proved with clinical trials, Therefore this study was undertaken to screen the native zebu cattle of Pulikulam breed in Tamil Nadu, India for the presence of A1 and A2 allele. Pulikulam cattle breed is mainly reared for bull raiding games and bull baiting. G. Srinivasan and T. Sathiamoorthy (2020) [17]. More than 500 to 1000 animals are being reared as a single herd in the breeding tracts traditionally in Madurai and Sivagangai districts of Tamil Nadu.

2. Materials and Methods

2.1 Experimental animals

A total of 100 numbers of Pulikulam cattle were chosen from Madurai (50 numbers) and Sivagangai districts (50 animals). In Madurai district the animals are being reared in herds and each herd consist a minimum of 200 animals to a maximum of 1000 cattle. But in Sivagangai district the animals are being reared in the house hold with minimum of two animals to maximum of 15 numbers in each household and it is reared mainly for their livelihood security. Especially in Madurai and Sivagangai districts rearing of pulikulam cattle is considered as pride for the owners and many families are rearing pulikulam cattle traditionally form generation after generation. This is the first report about A1A2 allele screening in Pulikulam cattle.

2.2 Blood collection and DNA extraction

10 ml of blood was collected aseptically from the jugular vein of each experimental animals with 2.7% EDTA and the Genomic DNA was isolated by phenol: chloroform extraction method as described by Sambrook and Russell (2001) [17].

2.3 Quality concentration and purity of DNA: The quality of Genomic DNA was checked for the presence of intact DNA by checking the isolated DNA on 0.8% agarose gel. The purity of the genomic DNA was checked with UV spectrophotometer by checking the optical density (OD) value at 260 and 280 nm. The samples having OD ratio (260 nm/ 280 nm) ranging from 1.8 to 2.0 were used for further experimental study. The concentration of DNA was calculated by using the following formula: DNA concentration = OD260 x (Dilution factor) x 50/ 1000. Finally the concentrated DNA was further diluted with dnase free water (Genei) at the concentration of 100 ng / µL make working solution and stored at-40 °C deep freezer for further use. For the amplification of PCR reaction two µl of diluted DNA (approximately 200 ng) was used.

2.4 Genotyping of beta casein gene

2.4.1 Allele specific PCR amplification: In order to identify the beta-casein variants, the following strategy was followed. The forward allele-specific primers were designed with adenine (A) at 3' terminal for A1 specific primer, corresponding to Thiamine (T), whereas Cytosine (C) at the 3' end of the A2 specific primer corresponding to Guanosine (G) and a common reverse primer were designed to amplify 840bp and 838bp products respectively for differentiating A1 variant from A2 as per Rahimi *et al.*, (2015) [16] For each sample the PCR reaction was carried out with two forward primers- A1 allele-specific Forward primer-1 (5' ATCCCTTCCCTGGACCCATCCA 3') and A2 allele-specific Forward primer -2 (5' CCCTTCCCTGGACCCATCCC3') with one Common reverse primer-(5'CCTTCTTAGGTTTGTTATTCTTAGCC 3') for amplifying the PCR products. The PCR reaction was carried out in a reaction volume of 25µl containing 15 µl of Ampliqon Red master mix with 1 µl of each forward primers and 1 µl of reverse primer and remaining 6 µl of nuclease free water with 2 µl of diluted DNA. The following PCR amplification were applied with one cycle of initial denaturation of 94 °C for 5 minutes followed by 35 cycles of 94 °C for 1 minute of denaturation, primer annealing at 58 °C for 1 minute and extension at 72 °C for 1 minute followed with final extension for 10 minutes at 72 °C in a Master cycler X 50 (Eppendorf) PCR machine. The amplified PCR products were electrophoresed in a 2% agarose gel with 100 bp DNA ladder and visualized under Gel documentation system (Bio Rad, USA) for identifying the amplified product of A1 or A2 or with A1and A2.

2.4.2 Statistical analysis

The gene (allele) and genotype frequencies were calculated by simple frequency calculations Falconer and Mackay, (1996) [5]. Effectiveness of allele incidence was evaluated using theoretical heterozygosity (Heexp) as per Nei (1973) [15], experimental heterozygosity (Heobs), expected homozygosity (E), effective number of alleles (ENA) as per Crow and Kimura (1970) [3]. Polymorphism information content value (PIC) was estimated according to Botstein *et al.*, (1980) [2].

3. Results

3.1 Gene and genotype frequencies

From this study it was observed that among the 100 animals 84 animals showed A2A2 (838bp) genotypes, 14 animals showed A1A2 (838 and 840bp) genotypes and two animals showed A1A1 (840bp) genotypes (Figure.1.). In Madurai district all the animals (50 animals) showed only A2A2 genotypes. In Sivagangai district all the three genotypes A1A1 (two animals), A1A2(14 animals) and A2A2(34 animals) genotypes were observed. The gene and genotypic frequencies at this locus have been depicted in Table 1.along with other population parameters. The observed heterozygosity, expected heterozygosity and polymorphic information content observed was 0.14, 0.164 and 0.150 respectively.

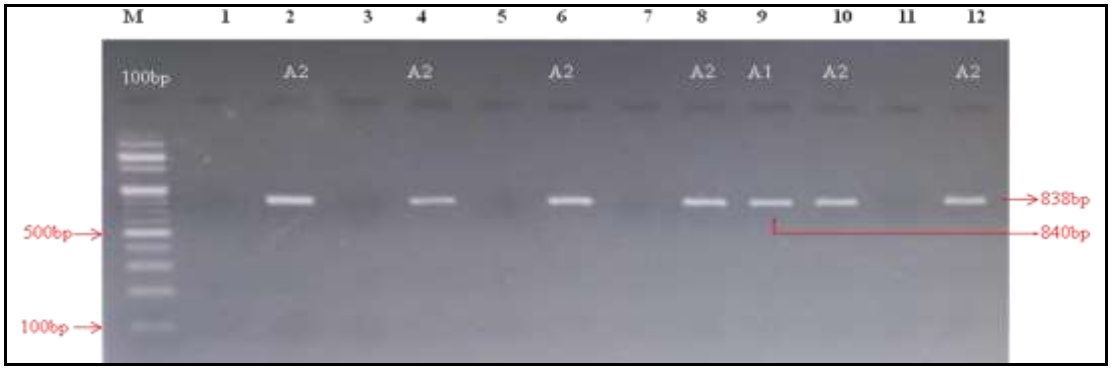

Fig 1: Representing the electrophoresis of allele specific PCR products in 2% Agarose gel
Lane: 2, 4, 6, 8, 10, 12 – A2 specific amplified PCR product
Lane: 1, 3, 5, 7, 11- A1 specific but not amplified.
Lane: 9 - A1 specific amplified PCR product

Table 1: Gene and Genotype frequencies along with population genetic indexes of A1/A2 variants in Pulikulam cattle.

Genotypes			Allele/Gene		Population genetic indexes		
A1A1	A1A2	A2A2	A1	A2	Ho	He	PIC
0.02 (2)	0.14(14)	0.84(84)	0.09	0.91	0.14	0.164	0.150

4. Discussion

In the present study it was an attempt to identify the polymorphism of β casein gene in Pulikulam cattle. The frequency of A2 allele was 0.91 and theA1 allele frequency was 0.09.this indicates that zebu cattle has higher frequency of A2allele.In the earlier research also it was observed the similar frequency of A2 allele in indigenous cattle in India was 0.94 in Ongole breed, 0.93 in Sahiwal breed 0.94 in Gir breed and 0.96 in Tharparkar breed of cattle. Ganguly *et al.* (2013) [7], Mir *et al.* (2014) [14] and Kumar *et al.* (2018) [11]. Polymorphism information content (PIC) is an indicator of degree of in formativeness of a marker and the genetic diversity normally ranges from 0 to 1. In the present study the observed value of PIC was found to be 0.1504 in Pulikulam cattle which shows a low genetic diversity.

5. Conclusion

In the past years the beta-casein polymorphism has attracted the common people as well as the researchers because of the connection between certain beta casein genetic variants and human health. In Madurai district all the animals showed only A2A2 genotypes. In Sivagangai district all the three genotypes A1A1,A1A2 and A2A2 genotypes were observed. This is mainly due to rearing of pulikulam cattle in Madurai district as herd and the breeding bulls are also maintained in the herd itself and natural mating is followed in the herd. But apart from the presence of A2 allele a low frequency of A1 allele is noticed in Sivagangai district this may be due to indiscriminate breeding of pulikulam cows with other cross bred animal during grazing in open areas or practicing of Artificial inseminations in pulikulam cattle with other breed semen with the interest of the farmers to increase the milk yield. More frequency of A2A2 allele in pulikulam cattle population (0.910) can be used as a proper guidance to be conveyed to the animal keepers for breeding A2A2 genotype and to identify and eliminate A1A1 genotype animals. Hence careful and planned mating may helps to eliminate the frequency of A1 allele in the population which may ensure good health care for the humans. Pulikulam cattle maintained traditionally in the herd exclusively exhibited the A2 genetic variant, resulting in the production of safer A2 milk for human consumption.

6. Acknowledgement

The authors are grateful to Tamil Nadu Livestock Development Agency (TNLDA) for providing necessary financial assistance to carry out the present investigation.

7. Conflict of Interest: No potential conflict of interest relevant to this article was reported.

8. References

1. Barnett MP, McNabb WC, Roy NC, Woodford KB, Clarke AJ. Dietary A1β-casein affects gastrointestinal transit time, dipeptidyl peptidase-4 activity, and inflammatory status relative to A2β-casein in Wistar rats. Int J Food Sci Nutr. 2014;65(6):720-727.
2. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphism. American Journal of Human Genetics. 1980;32:314-331.
3. Crow JF, Kimura M. An Introduction to Population Genetics Theory. Burgess Publishing, Minneapolis, 1970.
4. Elliott RB, Harris DP, Hill JP, Bibby NJ, Wasmuth HE. Type I (insulin dependent) diabetes mellitus and cow milk: casein variant consumption. Diabetologia. 1999;42:292-296.
5. Falconer DS, Mackay TFC. Introduction to quantitative genetics. 4th ed. London: Longman Group, 1996.
6. Farrell Jr, HM, Jimenez-Flores R, Bleck GT, Brown EM, Butler JE, Creamer LK, *et al*. Nomenclature of the proteins of cows' milk. Sixth revision. Journal of Dairy Science. 2004;87:1641-1674.
7. Ganguly I, Gaur GK, Singh U, Kumar S, Mann S. Beta-casein (CSN2) polymorphism in Ongole (Indian zebu) and Frieswal (HF×Sahiwal crossbred) cattle. Ind. J. Biotech. 2013;12:195-198.
8. Ho S, Woodford K, Kukuljan S, Pal S. Comparative effects of A1 versus A2 beta-casein on gastrointestinal measures: a blinded randomised cross-over pilot study. Eur J Clin Nutr. 2014;68:994-1000.
9. Jaiswal K, De S, Sarsavan A. 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. 2014;1:18-22.
10. Jianqin S, Leiming X, Lu X, Yelland GW, Ni J, Clarke AJ. Effects of milk containing only A2 beta casein versus milk containing both A1 and A2 beta casein proteins on gastrointestinal physiology, symptoms of discomfort, and cognitive behavior of people with self-reported intolerance to traditional cows' milk. Nutr J. 2016;15(1):35.
11. Kumar Sushil, Chauhan Anuj, Shukla Sanjeev Kumar, Singh Vir Ran. Molecular Characterization of A1/A2 Beta-Casein Alleles in Tharparkar and Gir Cattle. J Exp. Zool. India. 2018;21:403-405.
12. Laugesen M, Elliott R. Ischaemic heart disease, type 1 diabetes, and cow milk A1 beta-casein. New Zeal Med J. 2003;116:1-19.
13. McLachlan CN. Beta-casein A1, ischaemic heart disease mortality, and other illnesses. Med Hypotheses. 2001;56:262-272.
14. Mir Shahlla N, Ullah Obaid, Riazuddin Sheikh. Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. African Journal of Biotechnology. 2014;13(4):555-565.
15. Nei M. Analysis of gene diversity in subdivided populations. Proceedings of the National Academyof Sciences of the United States of America. 1973;70:3321-3323.
16. Rahimi Z, Gholami M, Rahimi Z, Yari K. Evaluation of beta-casein locus for detection of A1 and A2 alleles frequency using allele specific PCR in native cattle of Kerman shah, Iran Biharean Biologist. 2015;9(2):85-87.
17. Sambrook J, Russell DW. Molecular Cloning: ALaboratory Manual III. Cold Spring Harbour, Cold Spring Laboratory Press, NY. 2001.

18. Sun Z, Zhang Z, Wang X, Cade R, Elmer Z, Fregly M. Relation of beta-casomorphin to apnea in sudden infant death syndrome. Peptides. 2003;24:937-943.
19. Srinivasan, Sathiyamoorthy. Morphometric characteristics of Pulikulam Cattle breed in a nucleus herd.Journal of Entomology and Zoology Studies. 2020;8(3):1893-1895.
20. Tailford KA, Berry CL, Thomas AC, Campbell JH. A casein variant in cow's milk is atherogenic. Atherosclerosis. 2003;170:13-19.
21. Ul Haq MR, Kapila R, Shandilya UK, Kapila S. Impact of milk derivedβ-casomorphins on physiological functions and trends in research: a review. Int J Food Prop. 2014;17(8):1726-1741.