The Pharma Innovation Journal 2023; SP-12(11): 1761-1763

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 1761-1763

www.thepharmajournal.com
Received: 01-08-2023
Accepted: 07-09-2023

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Exon IV polymorphism of prolactin (PRL) gene in non-descript Gurez cattle of Gurez Valley of Kashmir

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Abstract
Prolactin is crucial for the growth of mammary glands, milk production, and milk protein gene expression. Principally produces lipids, lactose, and other significant milk constituents. It is a possible locus for quantitative traits and a genetic indicator of animal production traits. The current study screened local non-descript Gurezi cattle from Gurez Valley of Kashmir for Prolactin (PRL) gene by PCR-RFLP to explore potential genotypes of Gurez cattle. Gurezi cattle is an unexplored animal genetic resource that are exclusively found in the Gurez valley, situated in the Kishan Ganga basin in district of Bandipora. The population is well acclimated to the local agro-climatic conditions of area and reared primarily for milk and draught purpose by the Dardi tribes of Gurez Valley. On restriction digestion (Rsa I) of Prolactin gene (294 bp), three distinct banding patterns AA (a single band of 294 bp), Aa (three bands of 294/162/132 bp), and aa (two bands of 162/132 bp) were observed on gel (2.5%). The gene and genotype frequencies were estimated by using HWE formula. The population was found to be HWE and the genotype with higher frequency was found to be Aa, concluding that the Gurezi cattle shows polymorphism for PRL gene.

Keywords: Gurezi cattle, Gurez valley, prolactin, polymorphism, PCR-RFLP

Introduction
Prolactin (PRL) is a polypeptide hormone that is produced and secreted by certain cells in the anterior pituitary gland. The mammary gland has been shown to respond to prolactin hormone in a variety of ways, including development and growth (mammogenesis), milk production (lactogenesis), and the maintenance of galactopoiesis [1]. Additionally, it is principally in charge of producing lactose, lipids, and all the other important milk components [2]. The gene that codes for *PRL* is regarded as gene network's most crucial critical linkages that makes up the milk productivity hereditary component. Due to these qualities, it is a promising candidate gene for milk quality. PRL gene has been identified in close proximity to bovine Quantitative Trait Loci (QTL) on chromosome 23 (23q21) [3] around 10 kb and is comprised of 4 introns and 5 exons that code for 199 amino acids [4]. Restrictions Fragment Length Polymorphism (RFLP) has gained popularity as a marker for genetic characterization of *Bos indicus* with Polymerase Chain Reaction (PCR) [5-8]. Considering the significance of PRL gene for the production of milk, study's goal was to use PCR-RFLP to examine the PRL gene polymorphism in local non-descript Gurezi cattle of Gurez Valley in Kashmir. The Gurez cattle also known as local hill cattle or pahari cattle are a traditional population of cattle found in the Gurez Valley of Kashmir, India. These cattle are primarily raised by the Dardi tribes have medium-sized with a sturdy build. They have a distinct appearance with long, upward-pointing horns, which can be quite large in some individuals. These cattle are well adapted to the harsh climatic conditions of the Gurez Valley, which is known for its cold winters and heavy snowfall. They are hardy and can withstand extreme temperatures. Gurez cattle are primarily raised for milk production. While they may not produce as much milk as some other dairy breeds, their milk is known for its richness and quality.

Materials and Methods
Sampling: Using vacutainer tubes containing 0.5 M EDTA (pH 8.0), 8–10 ml of blood was drawn from 50 unrelated cattle from 27 separate villages of Gurez and Tulail valley (breeding tract) of district Bandipora (Fig 1). Samples were sent to the lab chilled to 4 °C in an ice boxes.

DNA Extraction: Using phenol/chloroform procedure, the DNA was extracted from whole blood [9]. DNA quality was assessed over a 0.8% agarose gel and O.D was calculated using a Spectrophotometer. A working dilution of 50 ng/µl concentration was used for amplification.

PCR amplification

Exon IV (294 bp) was amplified by using a pair of forward primer 5'CCAAATCCACTGAATTATGCTT3' and reverse primer 5'ACAGAAATCACCTCTCATTCA3' [10]. The total reaction volume for PCR was 25 µl, and each reaction contained 50 ng of each primer, 1U of *Taq* DNA polymerase, 200 M of dNTP, and 50 ng of template DNA in 10X buffer with $MgCl_2$. The cycles used were: initial denaturation at 94 °C for two minutes, followed by 36 cycles of denaturation at 94 °C for 30 seconds, annealing at 57 °C for 30 seconds, and extension for 45 seconds at 72 °C. The amplification was confirmed through 1.5% agarose gel electrophoresis. Bands were visualized using U.V transilluminator and documentation was done by Gel Doc system.

RFLP using *Rsa I* Restriction Enzyme

PCR products were digested with 5U of *Rsa I* (Thermo Scientific) at 37 °C for 1 hour. To analyze restricted fragments electrophoretically a 2.5% agarose gel was used. Manual scoring of the banding patterns was done using U.V transilluminator and documentation was done by Gel Doc system.

Statistical Analysis: The gene and genotype frequency was estimated using Hardy-Weinberg model using the formula $p^2 + 2pq + q^2 = 1$ while p = frequency of dominant allele and q is the frequency of recessive allele, p^2, 2pq and q^2 represent the genotypes.

Results and Discussion

A specific single band of 294 bp in length was produced by PCR amplification using a specific primer pair reported by Brym P. *et al*., 2005. Three unique banding patterns were observed on restriction digestion (*Rsa I)*, which were designated as AA (a single band of 294 bp), Aa (three bands of 294 bp/162 bp/132 bp), and aa (two bands of 162 bp/132 bp). Similar RFLP patterns in Zebu and exotic cattle [6], Kankrej [7], Jersey and Crossbred HF cows of dairy cattle of Kashmir [11], in Montebeliard cows [12] have been reported. In our investigation, Aa (0.48) genotype was shown to be the most prevalent genotype, followed by AA (0.28) and aa (0.24) genotypes. Similar findings were made with the Native cattle herds in Meghalaya (0.63) and Nagaland (0.50), in which AB genotype predominated with AA and BB having nearly comparable frequencies [13]. Similar findings were found in 23 native cow breeds of India, with a higher frequency of the AB genotype (0.58) and homozygous AA (0.22) and BB (0.20) in a comparable range [14]. Our study was also in agreement with native Indian breeds- Red Sindhi (0.62), Kankrej (0.62), and Gir (0.49) which also have greater AB genotype frequencies [15].

Table 1: Prolactin locus genotype and allele frequency determined by Rsa I -RFLP in Gurezi cattle

PRL-Rsa I genotype	Frequency	Allele Frequency	
		A	a
AA	0.28	0.52	0.48
Aa	0.48		
Aa	0.24		

Fig 1: Gurezi cattle distribution and sampling locations

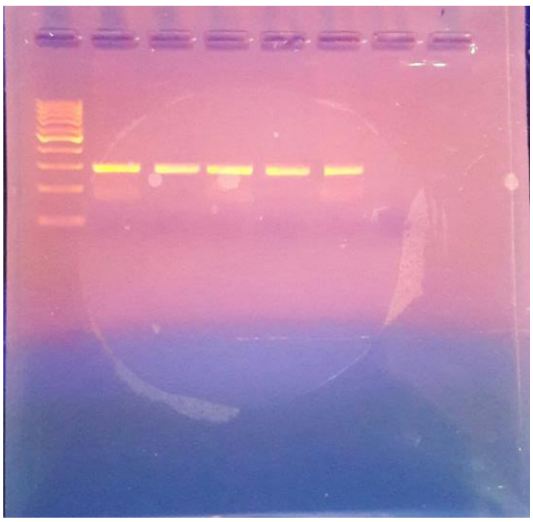

Plate 1: PRL gene Exon IV amplification (294 bp)

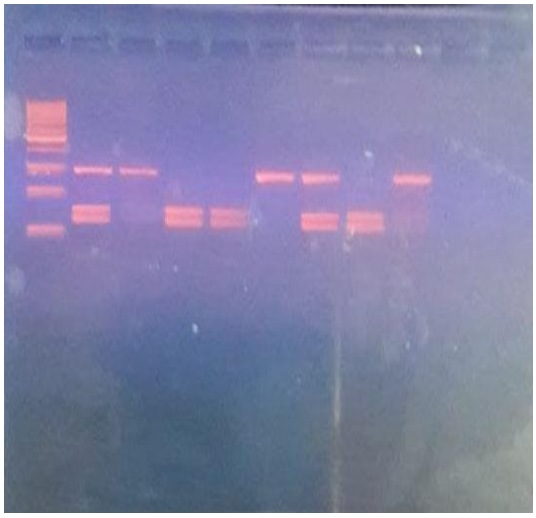

Plate 2: PRL gene polymorphism with Rsa I enzyme.

Two bands 162 and 132 bp: aa genotype; one band 294 bp: AA genotype; Aa genotype 162, 132, and 294 bp.

Conclusion

The different pattern found on RFLP study in the native local Gurezi cattle of district Bandipora of Kashmir is predictive of that this population is polymorphic for PRL gene.

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