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Prolactin gene polymorphism in indigenous and crossbred cattle of north east India

LALHRUAITLUANGI[1], CHUKHAM GOHAIN[2], KEYOLENU YORE[3], N SHYAMSANA SINGH[4], PRAVA MAYENGBAM[5] and T C TOLENKHOMBA[6]

Central Agricultural University, Selesih, Aizawl, Mizoram 796 014 India

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Prolactin (PRL) is one of the most versatile hormones of the pituitary gland in terms of its biological activities. The gene has been mapped on chromosome 23 by Hallerman *et al.* (1988). It consists of five exons and four introns encoding the 199–amino-acid mature protein (Camper *et al.* 1984). PRL plays extremely important roles in growth and development of the mammary glands, initiation and maintenance of lactation and also primarily responsible for the synthesis of milk proteins, lactose, lipids and all other major components of milk (Horseman *et al.* 1997). Therefore, bovine PRL gene is considered one of the most important key links in the gene network constituting the hereditary components of milk productivity, thus seems to be an excellent candidate gene for milk production traits (Patel and Chauhan 2017). Many studies had reported several polymorphic sites in PRL gene (Dybus *et al.* 2005, Kumar *et al*. 2017). The association of AA genotype of PRL gene with higher lactation milk yield was reported in American Swiss cattle (Alfonso *et al*. 2012), HF × Sahiwal (Singh *et al.* 2015) and Gir and Kankrej (Patel and Chauhan 2017). Singh *et al*. (2015) reported the correlation of BB genotype of PRL gene with lower somatic cell count in HF × Sahiwal cross.

The eight north east (NE) states of India contribute around 6.9% of total cattle population of the country (Livestock Census 2012). The indigenous cattle of north east India are mostly of non-descript type and mainly used for draft purpose. The present study aimed to determine the polymorphism of PRL gene in the indigenous cattle of north east India.

Blood samples were collected from indigenous cattle (*Bos indicus*) of 6 states, viz. Manipur, Mizoram, Nagaland, Tripura, Asom and Meghalaya and crossbred cattle of Mizoram. A total of 210 unrelated animals (30 each) of both sexes, irrespective of age were randomly selected from field(s) and farm(s). Genomic DNA was extracted using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) according to the instruction manual. The quantity and quality of DNA were checked with a NanoDrop MultiscanGo Spectrophotometer (Thermo Scientific, USA). A pair of PRL gene exon 3 specific primers (forward – 5' CGA GTC CTT ATG AGC TTG ATT CTT 3' and reverse -5'GCC TTC CAG AAG TCG TTT GTT TTC 3') were used for amplification (Alipanath *et al.* 2007). The PCR amplification was carried in a 25 µl of 10× PCR buffer, 2 mM of $MgCl_2$, 200 µM of each dNTPs, 5 pM each of primers, 2 U Taq DNA polymerase and 60 ng genomic DNA. The following cycles were applied: 94ºC for 5 min, followed by 35 cycles of 94ºC for 30 sec, 59ºC for 40 sec, 72ºC for 20 sec and final synthesis at 72ºC for 3 min. The amplified DNA was digested with *Rsa*I enzyme by incubating at 37ºC for 4 h. The digested products were analysed electrophoretically using 3% agarose gel in 0.5 × TAE containing 1.0 µM ethidium bromide. The bands were visualized under UV transilluminator and photographs were taken using Gel Doc system. The allelic and genotype frequencies calculation as well as the chi-square test to determine the possible deviations of genotype frequencies from expectation were carried out by using the Popgene32 software (Yeh *et al.* 1997).

In the PRL gene, point mutation arise from adenine to guanine (A103G) shift, that results in two alleles (A and B). The amino acid adenine that has at position 103 was denoted as the A allele. The mutation from adenine to guanine caused the formation of a restriction site for *Rsa*I restriction enzyme and this allele was denoted as B allele. The 156 bp fragment of exon 3 region of PRL gene digested with *Rsa*I revealed three genotypic patterns across all the studied population. The first pattern with an uncut single fragment of 156 bp was designated the AA genotype (absence of restriction site). The second pattern with two fragments (82 and 74 bp) was referred to as BB (presence of restriction site) and the third pattern with three fragments (156, 82 and 74 bp) was AB genotype (Fig. 1).

In indigenous cattle of Manipur, the heterozygous AB (0.40) and homozygous BB (0.40) were the most common genotypes (Table 1). In Mizoram indigenous cattle, BB genotype was the most frequent genotype (0.57) while AA

Present address: [1,2,3]Research Scholar (hruaiirhlenthlei16@gmail.com, drchukhamgohain@gmail.com, keyoyore@gmail.com), [4]Associate Professor and Head (nsagb@rediffmail.com), [6]Assistant Professor (drkhomba10@gmail.com), Department of Animal Genetics and Breeding; [3]Associate Professor (dr_prava@rediffmail.com), Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry.

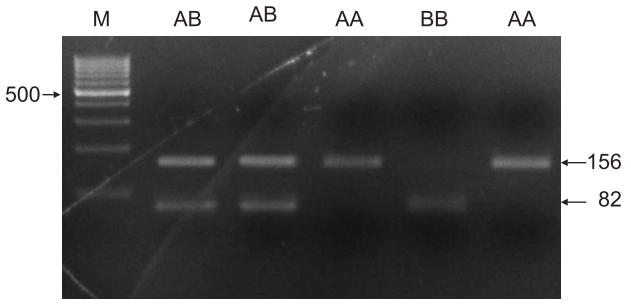

Fig. 1. Genotypes of PRL gene digested with *Rsa*I in 3% agarose gel

genotype was the lowest (0.10). AB genotype was observed as the most predominant genotype in indigenous cattle populations of Nagaland (0.50) and Meghalaya (0.63) while other two genotypes AA and BB were of almost similar frequencies. Sodhi *et al.* (2011) reported higher frequency of AB genotype (0.58) with homozygous AA (0.22) and BB (0.20) in a similar range in 23 Indian native cattle breeds. In other native breeds of India, higher frequencies of AB genotype in Kankrej (0.62), Gir (0.49) and Red Sindhi (0.62) were also observed (Kumari *et al.* 2008). Similar finding was observed by Patel and Chauhan (2017) who reported a higher frequency of AB genotype in Gir (0.83) and Kankrej (0.70).

Table 1. Genotypic frequency of exon 3 of PRL gene in local cattle of Manipur (MP), Mizoram (MZ), Nagaland (NL), Tripura (TR), Asom (AS) and Meghalaya (ML) and crossbred (CB) cattle

Genotype	Indigenous cattle						CB
	MP	MZ	NL	TR	AS	ML	
AA	0.20 (6)	0.10 (3)	0.30 (9)	0.40 (12)	0.60 (18)	0.17 (5)	0.10 (3)
AB	0.40 (12)	0.33 (10)	0.50 (15)	0.30 (9)	0.20 (6)	0.63 (19)	0.80 (24)
BB	0.40 (12)	0.57 (17)	0.20 (6)	0.30 (9)	0.20 (6)	0.20 (6)	0.10 (3)
Observed heterozygosity	0.40	0.33	0.20	0.30	0.20	0.63	0.80
Expected heterozygosity	0.48	0.39	0.42	0.50	0.42	0.50	0.50
χ^2 value	0.21^{NS}	0.02^{NS}	0.03^{NS}	0.08^{NS}	0.12^{NS}	0.03^{NS}	0.19^{NS}

NS, Not significant; Values within the parentheses are the number of animals.

On the contrary, in Tripura and Asom indigenous cattle populations, AA genotype was the most common (0.40 and 0.60) while AB and BB genotypes showed similar frequencies. Similar higher frequency of AA genotype (0.59) was reported in Russian Red Pied cattle (Alipanah *et al*. 2007). Skinkyte *et al*. (2005) also reported higher frequency of AA genotype in Luthianian Black and White (0.65) and Luthianian Red cattle (0.75). In present study, AB genotype (0.80) was markedly prominent than AA (0.10) and BB genotype (0.10) in crossbred (HF × indigenous) cattle population. However, higher frequency of AA genotype (0.59) in Indian Frieswal (HF × Sahiwal) was reported by Singh *et al.* (2015). The calculated Chi-square value revealed that all the six indigenous cattle populations were in confront to HWE with respect to exon 3 of PRL genotypes, which revealed the lack of selection pressure for milk traits. Whereas, crossbred cattle did not follow the equilibrium more likely due to introduction of genes from HF to the indigenous cattle. However, significant deviations from the HWE in the exon 3 PRL genotypes were observed in Kankrej and Gir cattle populations (Patel and Chauhan 2017). The allelic distribution of exon 3 PRL gene showed that A allele was the predominant allele in the indigenous cattle populations of Nagaland (0.55), Tripura (0.55) and Asom (0.70).

However, in indigenous cattle of Manipur (0.60) and Mizoram (0.73), B allele was the most frequent one (Table 2). Whereas, in Meghalaya indigenous and crossbred cattle, both alleles were present in equal frequencies. Kumari *et al.* (2008) observed that A allele was the most predominant allele in this same locus of PRL gene in various Indian native cattle breeds except in Red Sindhi and Red Kandhari similar to the present result in Nagaland, Tripura and Asom indigenous cattle. Findings pertaining to Meghalaya indigenous and crossbred cattle were in close agreement to the findings in Kankrej and Gir cattle in the allelic profile data of exon 3 PRL gene (Patel and Chauhan 2017). Patel and Chauhan (2017) also observed similar gene frequencies of these two alleles. The findings further suggested that the indigenous cattle of six states of north east India showed differences in their genetic structure with respect to the PRL gene exon 3 locus. This might be attributed to their isolated breeding for many generations due to geographical topography of the region.

Several investigators had reported the associations of PRL genotypes with the milk production traits in different breeds of cattle (Alfonso *et al.* 2012, Bukhari *et al*. 2013, Singh *et al.* 2015, Patel and Chauhan 2017). Their findings mainly suggested that AA genotype might be the suitable genotype for better milk traits. Therefore, the present findings on PRL gene polymorphism in the indigenous cattle of six states of north east India will be useful in formulating a suitable breeding programme for genetic selection of increase milk production. However, further research should be conducted on PRL gene polymorphism and their associations with milk production traits in these indigenous cattle populations.

Table 2. Allelic frequency of exon 3 locus of PRL gene in local cattle of Manipur, Mizoram, Nagaland, Tripura, Asom and Meghalaya, and crossbred cattle

Allele	Indigenous cattle of						CB
	MP	MZ	NL	TR	AS	ML	
A	0.40 (12)	0.27 (8)	0.55 (16)	0.55 (16)	0.70 (21)	0.48 (15)	0.50 (15)
B	0.60 (18)	0.73 (22)	0.45 (14)	0.45 (14)	0.30 (9)	0.52 (15)	0.50 (15)

In present study, observed and expected heterozygosity of PRL locus were calculated from allele frequencies, considering the population in Hardy-Weinberg equilibrium. Its unbiased estimate was calculated by taking the number of allele into account. The observed and expected heterozygosity of PRL gene locus were within Hardy-Weinberg expectation in all the populations as revealed by the chi-square values (Table 1).

The findings of A allele as the most common in the indigenous cattle of Nagaland, Tripura and Asom; B allele in Manipur and Mizoram, and both alleles in equal frequencies in indigenous cattle of Meghalaya and crossbred indicated differences in the distributions of allelic variants of PRL-*Rsa*I locus among the indigenous cattle populations. The populations conforming to equilibrium indicates lack of selection pressure in these indigenous cattle populations of North East India as most of the animals were let loose in the field for grazing and it was expected that random mating took place within the population.

SUMMARY

Bovine PRL gene is considered one of the important hereditary components of milk productivity, thus seems to be an excellent candidate gene for milk production traits. The study was conducted to study the distribution pattern of allelic variants at the prolactin-*Rsa*I locus in 210 indigenous cattle of north east India (viz. Manipur, Mizoram, Nagaland, Tripura, Asom and Meghalaya,) and crossbred cattle of Mizoram. PCR-RFLP genotyping of a 156 bp fragment of prolactin (PRL) in exon 3 revealed two different allelic variants. The predominant genotype (s) were AB (0.40) and BB (0.40) in indigenous cattle of Manipur, BB in Mizoram (0.57), AB in indigenous cattle of Nagaland (0.50) and Meghalaya (0.63). Whereas in Tripura and Asom indigenous cattle, AA was the most common genotype (0.40 and 0.60). However, AB (0.80) was markedly higher in crossbred (HF × indigenous) cattle. All the six indigenous cattle populations conforming to equilibrium indicated lack of selection pressure for PRL gene in these cattle population. The most frequent allele was A allele in the indigenous cattle of Nagaland (0.55), Tripura (0.55) and Asom (0.70) and B allele in indigenous cattle of Manipur (0.60) and Mizoram (0.73). Whereas, in Meghalaya indigenous and crossbred cattle, both alleles were present in equal frequencies. The findings of differences in the distributions of allelic and genotype variants of PRL-*Rsa*I locus among the indigenous cattle populations of six states of north east India, suggested the presence of scope for genetic selection for milk production traits.

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