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No Association of Prolactin Receptor (*PRLR*) Gene and litter size in Gaddi Goat Breed Reared under Migratory System in Western Himalayan Ranges of Himachal Pradesh

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ABSTRACT

Gaddi is the predominant Indian goat breed also known as "White Himalayan goat", constituting 60-65% of total goats in the state of Himachal Pradesh. The polymorphism of prolactin receptor (*PRLR*) gene was found to have relationship with prolificacy in goats. In present study, polymorphism of intron 2 region of *PRLR* gene was investigated in Gaddi goats (n = 89) using PCR-SSCP and DNA sequencing approach. PCR-SSCP assay of 176 bp amplicon of intron 2 region of *PRLR* gene revealed polymorphism with three types of genotypes viz., AA, AB and BB with genotypic frequencies as 0.31, 0.55 and 0.14, respectively. The allelic frequency of alleles A and B were 0.59 and 0.41, respectively in all the screened goat population. Genetic diversity analysis revealed the value of N_e, H_{obs}, H_{exp} and PIC were 1.96, 0.52, 0.49 and 0.37, respectively. The N_e and H_{obs} values also indicated that sufficient genetic variation exists at the studied locus. F_{IS} estimate was observed as -0.15 indicating heterozygous excess at studied locus. DNA sequencing of amplified product revealed one nucleotide mutation (T92C) in intron 2 region of *PRLR* gene. The mean litter size in AA, AB and AB genotypes were 1.27±0.12, 1.41±0.09 and 1.84±0.26, respectively. No significant (*P>0.05*) associations of *PRLR* genotypes with litter size were observed. Effect of season and parity were also found to be non-significant (*P>0.05*) on litter size. Consequently, the study on additional data based on more number of animals in diversified flock should be carried out for future association studies.

Keywords: Prolactin receptor, PCR-SSCP, Prolificacy, Gaddi goats, Migratory system

Small ruminants, sheep and goats, play a vital role not only in meeting increased human demand for animal origin protein foods but also in sustainability of overall farming system in general and livestock production system in particular. Sheep and goat production is a predominant livestock activity in harsh climatic regions of the country particularly in hilly areas and arid zones with the little arable agricultural land. Gaddi goat, also known as "White Himalayan goat", is the predominant Indian goat breed constituting 60-65% of total goats in the state of Himachal Pradesh (HP). It is reared both for wool and mutton production by migratory nomads/tribe "Gaddi" (Sankhyan *et al*., 2016). The breed is primarily reared in transhumant/migratory/pastoralist production system. Few small sized flocks (4-5 animals) are also reared under stationary, semi intensive system. Only pasture grazing without any supplementary feed is provided in migratory system. Goats contribute largely to the livelihoods of the livestock-keeping households of low and medium input farmers, many of whom have few resources except their small holdings and livestock. In addition, goats are important to the subsistence needs as they can supply meat, milk, fur and cashmere. Unfortunately, the genetic mechanism of caprine prolificacy remains to be explored. However, the tendency of twinning and triplet births is inherited and similar in both sheep and goats (Hua *et al*.,

2008). Several studies suggest that fecundity of goats is not linked to the same loci in Bone morphogenetic protein receptor type-IB (*BMPR-IB*) and Bone Morphogenetic Protein 15 (*BMP15*) as in sheep. From an animal breeding perspective, polymorphisms of the Prolactin receptor (*PRLR*) gene have been associated with productive and reproductive traits in goats, sheep, cattle and buffaloes (Jabbour and Critchley, 2001; Terman, 2005; Javed *et al*., 2011; Zhou *et al.*, 2011; Parihar *et al*., 2017). The *PRLR* gene is a member of the growth hormone/prolactin receptor gene family containing regions of identical sequences (Kelly *et al.*, 1991). Associations of *PRLR* polymorphisms with prolificacy in some sheep (Chu et al. 2007) and goat (Zhang *et al.*, 2007; Chu *et al.*, 2008; Di *et al.*, 2011) breeds have also been reported. Breeding and optimum management are important aspects contributing significantly to improve production efficiency of small ruminants. Till date, no report *PRLR* polymorphism is available on migratory Indian Gaddi goat breed. Therefore, the present study was carried out with the objective of identification of polymorphism at *PRLR* gene and its association with litter size in migratory Gaddi goats.

MATERIALS AND METHODS

Breeding tract of Gaddi goats lies in Chamba, Kangra, Mandi Kullu, Kinnaur and Lahaul and Spiti in Himachal Pradesh (HP) extending to adjoining areas of Jammu & Kashmir (J&K) and Uttarakhand. Geographically the breeding tract lies in Western Himalayan ranges at latitude 31º06'-33º05'N and longitude 74º54'-78º10'E, at altitude varying from 500-3800 meters above mean sea level (MSL). The study was conducted in five adopted flocks in different migratory route in the Himalayan ranges of HP (India) and the different migratory routes are presented in Fig. 1. The reproductive data recording was done only for goats although the flocks were mixed with sheep and goats. All the animals were tagged and identified and baseline data was generated for reproductive traits. Afterward, these flocks were monitored periodically over five years (2011-2016) for their reproductive performance and 89 samples were collected after screening of reproductive records from five adopted flocks. The genomic DNA was isolated by phenol-chloroform extraction procedure (Sambrook and Russsel, 2001). Part of intron 2 of *PRLR* gene was amplified using set of forward (5′-TGTCAGTAAGCGTCAGAGGGC-3′) and reverse (5′-GGCTGGTGGAAGGTCACTCTT-3′) primers (Di *et al*., 2011). For amplification, 25 ul of PCR reaction was prepared by adding 10 pmole of each primer, 100 µM of each dNTPs, 1.5mM $MgCl_2$, 10X PCR buffer, 100 ng DNA template and 0.5 Unit Taq DNA polymerase. The amplification was carried out using a thermal cycler (BioRad, USA) with the following conditions: initial denaturation of 5 min at 94◦C followed by 35 cycles of denaturation at 94◦C, annealing at 68◦C and extension at 72◦C each of 45 sec and lastly the final extension of 5 min at 72◦C.

Single Stranded Confirmation polymorphism (SSCP) assay

PRLR gene polymorphism in goat populations was studied using SSCP. PCR product (3 µl) was properly mixed with 15 µl formamide dye (95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5 M EDTA). The mixture was denatured at 95°C for 5 min and snapped cool on ice for 15 min. Finally mixture was run on 12% native PAGE (30:1; acrylamide and bis-acrylamide) with 5% glycerol. The electrophoresis was performed at 200 V for 10 hr. Subsequently, silver staining (0.2% silver nitrate) was used to identify different genotypes and allelic patterns. The representative genotypes belonging to different genotypes were subjected to Sanger sequencing after purification of amplified PCR products.

Genetic diversity analysis and test for Hardy-Weinberg Equilibrium

Population genetic indices, gene and genotypic frequencies, gene heterozygosity (He), polymorphism information content (PIC), effective allele numbers (Ne) and inbreeding estimate (F_{IS}) were calculated using Popgene 32 (ver1.32), Microsoft Windows-based freeware for population genetic analysis (Yeh *et al*,. 1999). Chi square (χ^2) analysis was applied to test population for genetic equilibrium utilizing Popgene32 software.

Association study of *PRLR* genotypes with litter size in Gaddi goats

The following fixed effects model was employed for analysis of litter size in Gaddi does and least squares mean was used for multiple comparison in litter size among different genotypes,

$$Y_{ijklm} = \mu + H_i + LS_j + P_k + G_l + e_{ijklm}$$

Where Y_{ijklm} is the observed phenotypic value for litter size; μ is population mean, H_i is the fixed effect of i^{th} herd, LS_j is the fixed effect of the j^{th} kidding season (j = 1, 2, 3, 4); P_k is the fixed effect of the k^{th} parity (k = 1, 2, 3); G_l is the fixed effect of the l^{th} genotype (l = 1, 2, 3), and e_{ijklm} is the random residual effect of each observation. Analysis was done using the general linear model procedure of SAS (ver 9.3) (SAS Institute Inc, Cary, NC, USA). Mean separation procedures were conducted using a least significant difference test.

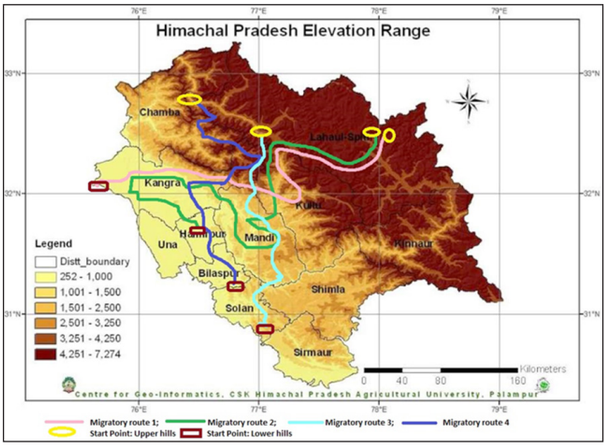

Fig. 1: Map depicting migratory routes of adopted Gaddi goat flocks for association studies

Source: All India Coordinated Research Project on Goats, Gaddi field unit Palampur (HP)

RESULTS AND DISCUSSION

PCR-SSCP assay of *PRLR* gene in Gaddi goats

Amplicon size of 176 bp was detected after amplification (Fig. 2). Similar amplicon sizes were reported by Di *et al*. (2011) in Jining Grey goat and Ji *et al*. (2016) in Haimen goat. The PCR-SSCP patterns observed for *PRLR* gene are presented in Fig. 3. PCR-SSCP assay demonstrated the presence of three type of genotypes viz., AA, AB and BB with two types of allele (A and B). The genotypic frequencies of AA, AB and BB genotypes were 0.31, 0.55 and 0.14, respectively. AB genotype was predominant genotype while BB genotype was having least frequency. The allelic frequency of alleles A and B were 0.59 and 0.41, respectively in screened goat population. Di *et al*. (2011) reported allelic frequency of A and B allele ranging from 0.78-0.93 and 0.06-0.25, respectively in five exotic/Indian goat breeds. Ji *et al*. (2016) in Haimen goat reported 2 alleles with gene frequencies of 0.79 and 0.21, respectively.

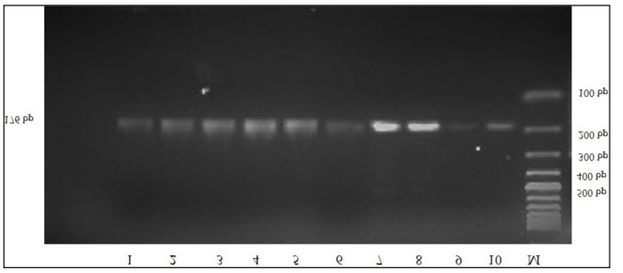

Fig. 2: PCR products representing amplification of intron 2 region of *PRLR* gene, in Gaddi goats. Lane M: 100 bp DNA ladder. Lanes 1 -10: 176 bp PCR products

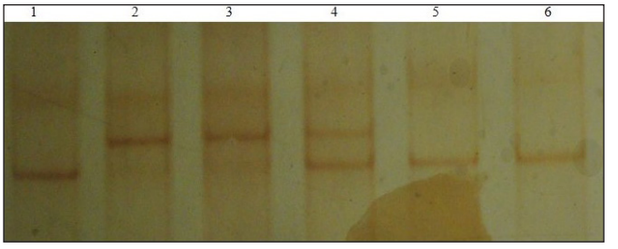

Fig. 3: PCR-SSCP patterns for intron 2 region of PRLR genein Gaddi goats. Lane 1, 5 and 6: AA pattern; Lane 2, 3: BB pattern; lane 4: AB pattern

Genetic diversity analysis and test for Hardy-Weinberg equilibrium

The effective number of alleles (N_e), observed heterozygosity (H_{obs}), expected hetrozygosity (H_{exp}) and polymorphic information content (PIC) values were estimated as 1.96, 0.52, 0.49 and 0.37, respectively in screened Gaddi goat population. The screened population was medially polymorphic 0.25 < PIC < 0.50 based on the PIC value. The N_e and H_{obs} values also indicated that sufficient genetic variation exists at the studied locus. Inbreeding coefficient (F_{IS}) estimate was observed as -0.15 indicating heterozygous excess at studied locus. Ji *et al*. (2016) in Haimen goat reported Ne, Hobs and PIC values as 1.49, 0.33 and 0.28, respectively, which were lower than those observed in present study. Chi square (χ^2) analysis

revealed that $\chi^2_{(cal)} < \chi^2_{(tab)}$ at 5% level of significance and 1 d.f. indicating that screened population of Gaddi goats was found in Hardy-Weinberg Equilibrium (HWE). However, Ji *et al*. (2016) reported an unbalanced HWE based on *PRLR* locus in Haimen goats.

DNA sequencing and analysis

Eighteen representative samples of different genotypes as revealed by PCR-SSCP were sequenced after purification of respective PCR product. Sequencing of amplicon revealed one nucleotide mutation (T92C) in intron 2 region of *PRLR* gene, which were similar to those reported by Di *et al*. (2011) and Ji *et al*. (2016) in Chinese goat population. The chromatogram representing the substitution is depicted in Fig 4.

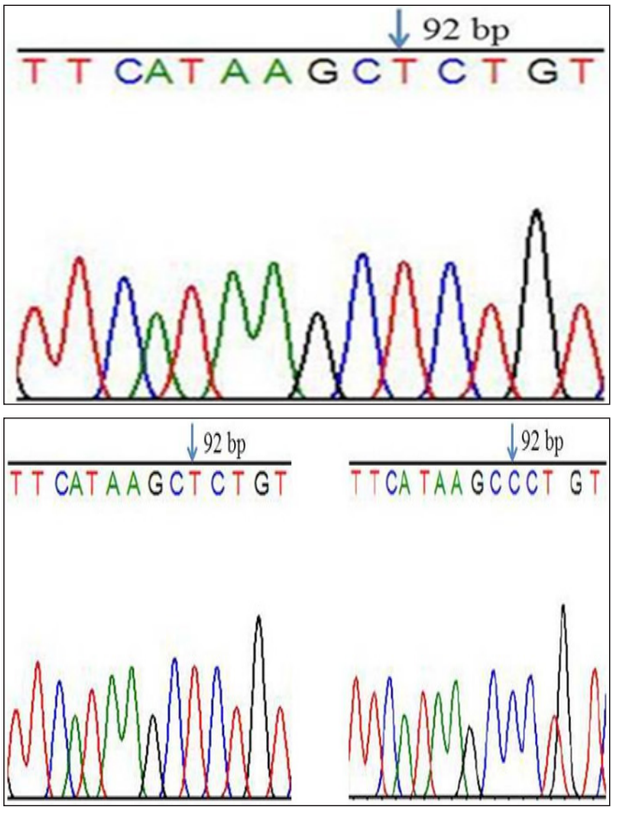

Fig. 4: Chromatogram representing T/C substitution in PRLR gene, intron 2 as detected by PCR-SSCP in Gaddi goats

Sequence alignment for wild type and mutant alleles corresponding to reference sequence retrieved from NCBI database using MegAlign programme of DNASTAR software is also confirmed presence of T/C base change at 92 bp. Apart from this, sequence analysis of representative samples also revealed one additional base change (C/T) at 144 bp.

Litter size and effect of *PRLR* gene polymorphism on litter size

The least squares means and standard errors of means (SEM) for litter size classified according to genotype, season and parity are presented in Table 2. The mean litter sizes in summer, winter and monsoon were 1.44±0.25 1.54±0.17 and 1.61±0.19, respectively. The parity wise litter sizes were 1.52±0.11, 1.44±0.18 and 1.59±0.10 for >3, 4-6 and >6 parity groups, respectively. Effect of season and parity were found to be non-significant (*P>0.05*). Non significant effect of parity on litter size was also reported in Sudanese Nubian (El-Hassan *et al*., 2009) and Markhoz goats (Shokrollahi and Morammazi, 2017). Similar to present investigation non significant effect of season on litter size was also reported by An *et al*., 2013 in Chinese goat breeds.

The mean litter sizes in AA, AB and AB genotypes were 1.27±0.12, 1.41±0.09 and 1.84±0.26, respectively. No significant (*P>0.05*) associations of genotype were observed with litter size. However, Di *et al*. (2011) observed significant association of intron 2 of *PRLR* gene with prolificacy in Jining grey goats. Ji *et al*. (2016) reported that SNP (T95C), corresponding to that observed in present study had no significant effect on litter size in Chinese goat populations, however, they reported significant association for another SNP (A35G), which was not detected in present study. Li *et al*. (2010) also reported that variations of *PRLR* gene intron 2 correlated significantly with litter size in Haimen goat and concluded that *PRLR* gene could be used as a candidate genetic marker for fecundity in goat. Absence of association in studied population could be attributed to the limited sample size, thus future studies based on more number of animals could be planned. Another approach could be to focus on other gene/loci for studying the prolificacy in migratory Gaddi goats since quantitative traits are regulated by large number of genes and are also affected by the interaction of these genes, so varied effect of a candidate gene associated with a particular trait in a population is observed and another loci could also be screened for prolificacy studies.

Table 1: Measures of genetic diversity and test of equilibrium for *PRLR* gene, intron 2 in Gaddi goats

Genotypic freq.		Allele freq.		Ne	H_{obs}	H_{exp}	PIC	F_{IS}	Chi square
Genotype	Freq.	Allele	Freq.						
AA	0.31 (28)	A	0.59	1.96	0.52	0.49	0.37	-0.15	0.23
AB	0.55 (49)	B	0.41						($P= 0.63^{NS}$)
BB	0.14 (12)								(d.f=1)

No: observed no of allele; Ne: effective no of alleles; He: Heterozygosity; PIC; Polymorphic information content: F_{IS}: Fixation index.

Table 2: Least squares means and standard error of means for litter size of different in Gaddi goats

Effect		Number	Litter size
Genotypes	AA	28	1.27±0.12
	AB	49	1.41±0.09
	BB	12	1.84±0.26
F-value =0.97; P>F=0.3837			
Season	Summer	15	1.44±0.25
	Winter	45	1.54±0.17
	Monsoon	29	1.61±0.19
F-value = 0.13; P>F=0.8744			
Parity	<3	35	1.52±0.11
	4-6	14	1.44±0.18
	>6	40	1.59±0.10
F-value = 0.32; P>F=0.7282			

CONCLUSION

In the present study, PCR-SSCP assay revealed polymorphism in the amplified product of the intron 2 of prolactin gene in migratory Gaddi goat population. DNA sequencing confirmed one nucleotide mutation (T92C) in intron 2 region of *PRLR* gene with allelic frequency of alleles A and B as 0.59 and 0.41, respectively. However, in present study no significant association were found at *PRLR* genotype with litter size in screened Gaddi goats. Effect of season and parity were also found to be non-significant on litter size. Therefore, the study on additional data based on more number of animals in diversified flock should be carried out for further association studies.

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