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Genotyping of the polymorphism within exon 3 of prolactin gene in various dairy breeds by PCR RFLP (Brief report)

(Genotypisierung des Polymorphismus im Exon 3 des Prolaktin Gens bei verschiedenen Milchkuhrassen mittels PCR RFLP)

Background: In mammals, especially dairy cattle the prolactin has important functions like the development of mammary gland affecting milk yield and composition. It has been mapped to chromosome 23 in Bovine (HALLERMAN et al., 1988). A silent A→G transition mutation at the codon for amino acid 103 in exon 3 of bovine prolactin (*bPRL*) gene gives rise to a polymorphic *Rsa* I site, has become a popular genetic marker used for genetic characterization of cattle populations by means of PCR-RFLP (MITRA et al., 1995; CHRENEK et al., 1998; DYBUS, 2005). The present study reports on the genotype frequencies observed in various *Bos taurus* and *Bos indicus* dairy cattle breeds.

Procedures:

Primer sequences:

sense primer: 5′–CGA GTC CTT ATG AGC TTG ATT CTT–3′

antisense primer: 5′–GCC TTC CAG AAG TCG TTT GTT TTC–3′

PCR-RFLP analysis:

The DNA was extracted from blood samples collected from cattle, mainly bulls, of exotic and indigenous breeds stationed at locations across the country (Table). The PCR mixture contained 1X PCR buffer, 0.4 mM dNTPs, 1 Unit of *Taq* DNA polymerase, 20 pM each of sense and antisense primer, 100 ng of DNA, 2.5 mM $MgCl_2$ in a final volume of 25 µl. The PCR reaction included the following steps, initial denaturation of 2 min at 94 ℃ followed by 35 cycles, each comprising 1 min at 94 ℃, 1 min at 65 ℃ and 0.5 min at 72 ℃ and final extension of 10 min at 72 ℃. The amplified product was digested by using *Rsa* I at 37 ℃ for overnight. The digested product was visualized on 4 % agarose gel.

Results: The genotyping procedure revealed three patterns of fragments of 156 bp (allele A) and 82 and 74 bp (allele B). Genotype frequencies and allelic frequencies of different breeds of cattle are presented in the Table 1. In a total of 501 animals of different breeds, the AA-genotype frequency (0.55) was predominant over the AB- (0.39) and the BB-genotype frequency (0.06). The A-allele was more frequent in all breeds except Red Sindhi and Red Kandhari (n=1). The finding are in line with observations made in Vechur cattle (ARAVINDAKSHAN et al., 2004), Polish Black and White (DYBUS, 2002) and in Holstein Friesian (0.90) (CHRENEK et al., 1998; DYBUS, 2002). Genotype and allele frequencies of indigenous cattle breeds are essentially similar to those obtained in exotic breeds selected for milk yield and milk fat content.

Table 1
Numbers of genotypes, genotype and allelic frequency of exotic and zebu cattles

Animal breed	No. of animals	No. of Genotypes/Genotype frequency AA	BB	AB	Allelic frequency A	B
Exotic cattle						
Holstein Friesian	223	180 (0.80)		43 (0.20)	0.90	0.10
Jersey	143	32 (0.22)	18 (0.13)	93 (0.65)	0.55	0.45
Sub total	366	212 (0.58)	18 (0.05)	136 (0.37)	0.77	0.23
Zebu cattle						
Sahiwal	13	10 (0.77)		3 (0.23)	0.88	0.12
Khillari	13	13 (1.00)			1.00	–
Ongole	5	4 (0.80)		1 (0.20)	0.90	0.10
Kankrej	26	8 (0.31)	2 (0.07)	16 (0.62)	0.60	0.40
Gir	41	15 (0.37)	6 (0.14)	20 (0.49)	0.61	0.39
Red Sindhi	26	4 (0.15)	6 (0.23)	16 (0.62)	0.46	0.54
Hariana	7	6 (0.86)		1 (0.14)	0.93	0.07
Red Kandhari	1		1 (1.00)			1.00
Dangi	1			1 (1.00)	0.50	0.50
Deoni	1	1 (1.00)			1.00	
Tharparkar	1	1 (1.00)			1.00	
Sub total	135	62 (0.46)	15 (0.11)	58 (0.43)	0.67	0.33
Grand total	501	274 (0.55)	33 (0.06)	194 (0.39)	0.74	0.26

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