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Association of MHC pattern and faecal egg counts in Bandur sheep

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The Bandur sheep has evolved over many generations of natural selection and is known for its high disease resistance (Nimbkar *et al.* 2000). Characterization and conservation of native Bandur sheep germplasm for disease resistance trait has become a necessity in view of increasing anthelmintic resistance in parasites (Swarnkar *et al.* 2001, Yadav and Garg 2004). The MHC is one of the candidate genes (Sayers and Sweeney 2005) that plays an important role in immune response (Lewin *et al.* 1991) and was found to be an ideal genetic marker in developing disease resistant flocks (Stear and Murray 1994, Amarante and Amarante 2003).

The high genetic variability in ovine MHC DRB region (Konnai *et al.* 2003, Sun *et al.* 2003) controls the differences in immune responses against disease (Nagaoka *et al.* 1999). It encodes the major part of antigen binding site (Dukkipati *et al.* 2006) and may influence the production of antibody during nematode infestation (Outteridge *et al.* 1996). PCR RFLP using a combination of restriction enzymes is a powerful tool for the detection of Ovar MHC DRB alleles (Konnai *et al.* 2003). Faecal egg count is being used extensively in sheep to understand the nature of genetic regulation of immune response and to identify its association with genetic resistance (Sayers *et al.* 2005).

Significant association between ovine MHC DRB alleles and faecal egg counts was observed by many workers Schwaiger *et al.* (1995), Buitkamp *et al.* (1996), Feichtlbauer *et al.* (1996) and Charon *et al.* (2002). Certain MHC alleles were found to be associated with low juvenile survival and high level of parasitism (Paterson *et al.* 1998).

The blood and faecal samples from Bandur sheep population were collected at random from unrelated individuals. Samples (63) were collected from villages of Mandya district of Karnataka, Bandur sheep breeding farm, Mandya and sheep farm of Department of Livestock Production and Management, Veterinary College, Bangalore. DNA was extracted from whole blood using standard protocol of high salt procedure (Miller *et al.*1988). The concentration and purity was adjudged on agarose gel and UV spectrophotometer. The quality of DNA was checked on 1% agarose gel.

The PCR-RFLP data were generated for ovine MHC DRB region for 63 samples using restriction enzymes *Hae*III and *Rsa*I. The PCR conditions were standardized for the primers LA31 and LA32 selected for the study. Forty cycles of amplifications were carried out at an annealing temperature of 62°C. The PCR products, i.e. 304 bp lengths, were tested on 1.75 % agarose with standard DNA marker. The restriction enzyme digestion of PCR products was carried out overnight with 10 units of *Hae*III and *Rsa*I at 37°C. The digested product was run on 3% agarose gel with standard DNA marker for 2 h. Faecal egg counts were done for each sample according to McMaster method (Coles *et al.* 1992).

Test of significance for the association of different MHC patterns with faecal egg counts were computed using a one-way analysis of variance of the Minitab Statistical Software (Minitab 1996). Faecal egg count values were transformed by square root transformation to make the values normal.

A 304 bp of OLA-DRB region fragment was amplified by LA31 and LA32 primers in all the animals studied. Eight and thirteen different MHC DRB patterns were detected with

Table 1. Different allelic patterns (bp) detected with *Hae* III restriction enzyme

Allele type	*Hae* III pattern	No. of animals
'a'	150, 115, 39	8
'b'	135, 109, 60	6
'c'	208, 96	23
'd'	208, 182, 135, 83	4
'e'	262, 208, 115, 23	5
'f'	175, 93, 72	3
'g'	175, 91, 38	5
'h'	247, 186, 175	9

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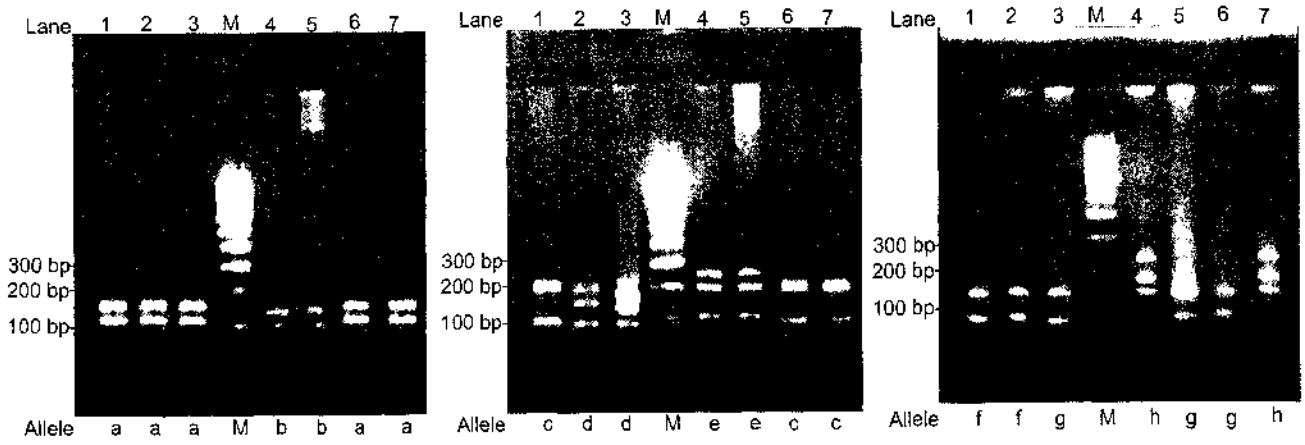

Fig 1. PCR-RFLP patterns of OLA-DRB exon -2-intron-2 region by *Hae* III restriction enzyme.

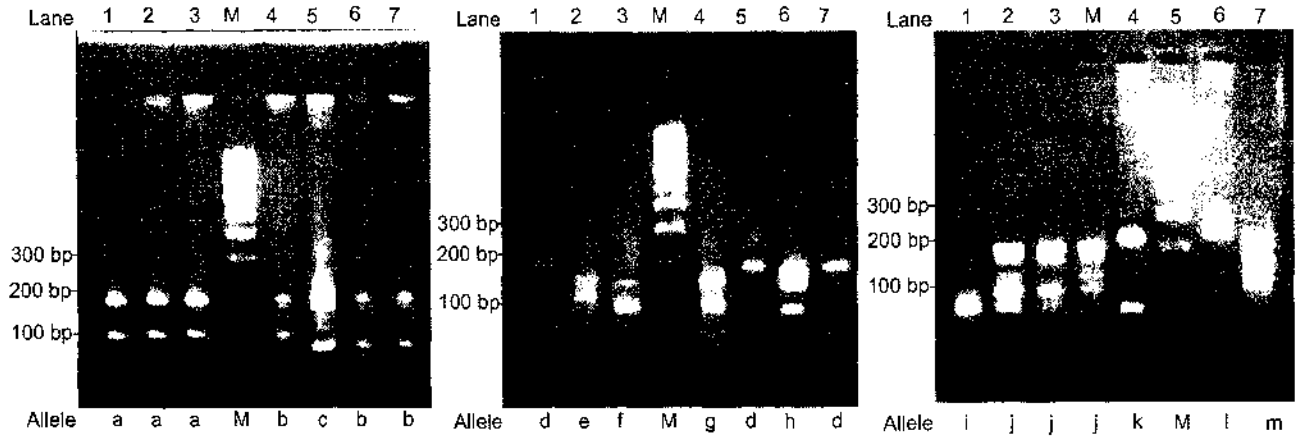

Fig 2. PCR-RFLP patterns of OLA-DRB exon -2-intron-2 region by *Rsa* I restriction enzyme.

*Hae*III and *Rsa*I restriction enzyme respectively (Figs 1, 2). Of the eight allelic patterns detected by *Hae*III, 'c' was dominant followed by 'h' and 'a' (Table 1) with frequencies of 36.50, 14.28 and 12.69 %, respectively (Table 3). The frequency of allelic pattern 'a' was the highest followed by 'k' and 'm' detected by *Rsa*I (Table 2). Since, the animals

Table 2. Different allelic patterns (bp) detected with *Rsa* I restriction enzyme

Allele type	*Hae* III pattern	No. of animals
'a'	177, 107, 20	12
'b'	179, 99, 26	3
'c'	179, 167, 95	1
'd'	184, 79, 41	6
'e'	154, 124, 26	4
'f'	151, 109, 44	2
'g'	157, 112, 35	5
'h'	181, 144, 102	3
'i'	160, 88, 56	4
'j'	184, 160, 108, 88, 68	5
'k'	216, 88	9
'l'	238, 66	2
'm'	208, 160, 120	7

Table 3. Frequency of allelic patterns of the OLA-DRB gene detected by *Hae*III and *Rsa*I enzymes

Allele type	Frequency (%)	
	Hae III	*Rsa* I
'a'	12.69	19.04
'b'	9.52	4.76
'c'	36.50	1.58
'd'	6.34	9.52
'e'	7.93	6.35
'f'	4.76	3.17
'g'	7.93	7.93
'h'	14.28	4.76
'i'		6.35
'j'		7.93
'k'		14.28
'l'		3.17
'm'		11.11

were randomly selected, it was difficult to characterize the patterns as homozygote or heterozygote. The restriction patterns were identified as homozygotes and heterozygotes based on the sum of the fragment sizes. With *Hae*III restriction enzyme, allelic patterns 'd', 'e', 'f' and 'h' were designated as heterozygotes and patterns 'a', 'b', 'c' and 'g' were considered as homozygote. *Rsa*I detected patterns 'a', 'b', 'd', 'e', 'f', 'g', 'i', 'k' and 'l' as homozygotes and 'c', 'h' 'j' and 'm' as heterozygotes. *Rsa*I detected more allelic patterns as compared to *Hae*III restriction enzyme (Sigurdardottir *et al.* 1991, Van Eijk *et al.* 1992).

Table 4. Mean square root transformed [Y=sqrt(X + ½)] faecal egg count (epg)±SD with different allelic patterns of *Hae*III enzyme (P<0.10)

Allelic pattern	N	Mean	SD
'a'	8	19	10
'b'	6	22	16
'c'	23	26	22
'd'	4	32	36
'e'	5	19	12
'f'	3	19	6.4
'g'	5	25	12
'h'	9	29	24

NS; CV, 83.93; SE, 2.60.

Table 5. Mean square root transformed [Y=sqrt (X+ ½)] faecal egg count (epg)±SD with different allelic pattern of *Rsa*I enzyme (P≤ 0.10)

Allelic pattern	N	Mean	SD
'a'	12	22	16
'b'	3	17	2.9
'c'	1	36	0
'd'	6	34	29
'e'	4	32	27
'f'	2	30	8.2
'g'	5	40	37
'h'	3	15	1.8
'i'	4	23	11
'j'	5	17	4.7
'k'	9	25	26
'l'	2	14	0
'm'	7	18	7.3

NS; CV, 79.12; SE, 2.39.

The mean faecal egg counts were observed as 99 epg, with a range from 0 to 8100. The epg values were subjected to square root transformation to reduce the heterogeneity and to make the data normally distributed. The association of different allelic patterns, detected by both enzymes, with faecal egg count was nonsignificant with a coefficient of variation (CV) of 83.93 and 79.12 for *Hae*III and *Rsa*I restriction enzymes, respectively (Tables 4, 5).

The high genetic variability present at MHC DRB locus is confirmed in Bandur sheep using PCR RFLP technique, which detected 21 alleles (Konnai *et al.* 2003, Yunfang *et al.* 2004), though this study could not detected any significant association between MHC DRB alleles and faecal egg count. The study is in agreement with Blattman *et al.* (1993) and Crawford *et al.* (1997) who also observed no significant association between any MHC DRB alleles and faecal egg count. This study detected variation only in DRB exon II region and it might be possible that any other region may influence the resistance to parasitic infection (Buitkamp *et al.* 1996). The inability to detect any association between DRB alleles and faecal egg count in Bandur sheep may be attributable to the different allelic profile at different MHC locus (Sayers *et al.* 2005).

The age of the animals could have significant effect on the expression of MHC genes and ultimately on the resistance or susceptibility to parasitic infestation (Schwaiger *et al.* 1995, Paterson *et al.* 1998). The inheritance of polygenes may also influence and create spurious relationship between trait and MHC pattern (Blattman *et al.* 1993). This study reinforces the importance of statistical model that account for family structure, multiple comparison and challenge study with particular parasite (Buitkamp *et al.* 1996).

SUMMARY

The present study was conducted to analyze the genetic variation of Bandur sheep in the OLA-DRB region of major histocompatibility (MHC) gene by using PCR-RFLP technique. and the association of its allelic types with faecal load of helminth eggs.

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