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Haemoglobin polymorphism in Gir crossbred cattle

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ABSTRACT : Haemoglobin polymorphism was investigated using blood sample of 141 Gir crossbred cattle including 80 half breed (50% Holstein Friesian × 50% Gir) and 61 triple crossbred (50% Holstein Friesian + 25% Jersey + 25% Gir) belonging to Research cum-Development Project, M.P.K.V., Rahuri (M.S.). Gir crossbred cattle were typed for haemoglobin using the electrophoretic technique (PAGE). Haemoglobin was polymorphic among the Gir crossbred cattle. The three phenotypes were observed viz., Hb AA, Hb AB and Hb BB in all the Gir crossbred cattle indicated the presence of two co-dominants alleles Hb^A and Hb^B controlling the occurrence of these three phenotypes. The heterozygous phenotypes Hb AB was most frequent, followed by homozygous phenotypes Hb AA and Hb BB. The gene frequency of Hb^A allele was more than Hb^B allele in half bred and triple crosses as well as overall Gir crossbred population. The electrophoretograms of haemoglobin at different ages of animal did not exhibit any change in the banding pattern/mobility of bands. The Chi-square (χ^2) test revealed the population to be under Hardy-Weinberg equilibrium with Hb^A and Hb^B genes.

KEY WORDS : Crossbred cattle, Gene frequency, Gir, Haemoglobin polymorphism

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INTRODUCTION

The bovine haemoglobin was first demonstrated by Cabbanes and Serain (1955). Several workers have reported the significant association between these haemoglobin types and economic traits in various exotic and Indian breeds. The existence of such association will help to select animals at very early age. Also, various crossbreeding programmes with different exotic inheritance were taken up in India and the frequency distribution of haemoglobin types in some of these crosses is not yet known. Hence, the present study was undertaken to study the haemoglobin polymorphism in Gir crossbred cattle which is one of the principal Zebu or *Bos indicus* breeds in India and is used for both dairy and beef production. It has been also used locally in the improvement of Red Sindhi, Sahiwal and Brahman breeds in North America.

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MATERIALS AND METHODS

The investigation on haemoglobin polymorphism in Gir crossbred cattle was carried out at Research-cum Development Project on Cattle, M.P.K.V., Rahuri (M.S.). The crossbred cattle included 80 animals of 50 per cent Holstein Friesian + 50 per cent Gir (IFG) and 61animals of 50 per cent Holstein Friesian + 25 per cent Jersey + 25 per cent Gir (IFJG). Haemolysate were prepared from the blood sample collected from Gir crossbred animals belonging to 0-3, 3-6, 6-12, and above 12 months of age groups as per the method described by Beherent (1957) for haemoglobin typing.

The polymorphic variants of haemoglobin, was done by vertical slab gel electrophoresis in polyacrylamide gel (PAGE) using a discontinuous buffer system at low temperature (4 - 8°C) with slight modification (Davis, 1964). Resolving gel with pH 8.8 were prepared by weighing 12.114 g tris-hydroxymethyl aminomethane in 80 ml distilled water and adding 1 N HCl drop wise to make the final volume 100 ml. Similarly, stacking gel with pH 6.8 was prepared with same chemicals. The electrophoresis was conducted initially at 30 mA till the samples migrated into running gel and subsequently at 50 mA at constant voltage. For separation of the haemoglobin bands electrophoretic run was continued for 6-8 hours. Staining of the haemoglobin bands was done in rinising solution of 1 per

cent amido black. Haemoglobin types were identified as Hb BB, Hb AA and Hb AB, respectively on the basis of migration rate as fast, slow and combination of two distinct bands.

The gene frequencies of haemoglobin were calculated by direct counting method (Li, 1955). The Hardy-Weinberg equilibrium was tested by chi-square method.

RESULTS AND DISCUSSION

The single slow moving band, a single fast moving band and a heterotype having two bands corresponding to the slow and fast moving band were observed in the blood samples of Gir crossbred cattle based on the relative mobility of the bands. These slow, fast and heterotype bands were designated as Hb AA, Hb BB and Hb AB, respectively (Fig. 1), which indicated the presence of two co-dominants alleles (Hb^A and Hb^B) controlling the occurrence of these three haemoglobin phenotypes. The present findings were in close agreement with the earlier reports (Jain *et al.,* 1995; Deshpande and Sawant, 1998; Harikrishnaraj *et al.,*(2001*),* Di Vito *et al.,* 2002 and Apparnavar *et al.,* 2003). However, haemoglobin variants recorded in Indian Zebu and African breeds viz. Hb-Khillar and Hb-X by Naik and Sanghvi (1964) were not found in Gir crossbred cattle. The absence of these rare variants in Gir crossbred cattle were suggested that, either it had very low frequency or was not present in the gene pool of foundation stock of Gir herd. Further, these results were supported by the absence of these variants in the exotic breeds of European origin which have been used for generating the crossbreds cattle (Jain *et al.,* 1995; Di Vitro *et al. ,* 2002).

The gene frequencies of Hb^A and Hb^B exhibit a definite pattern wherein, Hb^A allele was more frequent in all the genetic groups and overall Gir crossbreds than Hb^B allele. The gene frequencies for Hb^A and Hb^B alleles were 0.5437, 0.4563 in IFG 0.5738, 0.4262 in IFJG and 0.5567 and 0.4433 in overall Gir crossbreds (Table 1). However, these results were compared well in Zebu and Jersey crossbred cattle in India (Rao *et al.,* 1973). Similar results were recorded by Kirmani *et al.* (1993) and Senapati *et al.* (1997) for the gene frequency of Hb^A alleles in Sahiwal and Haryana crosses with Jersey and Holstein Friesian.

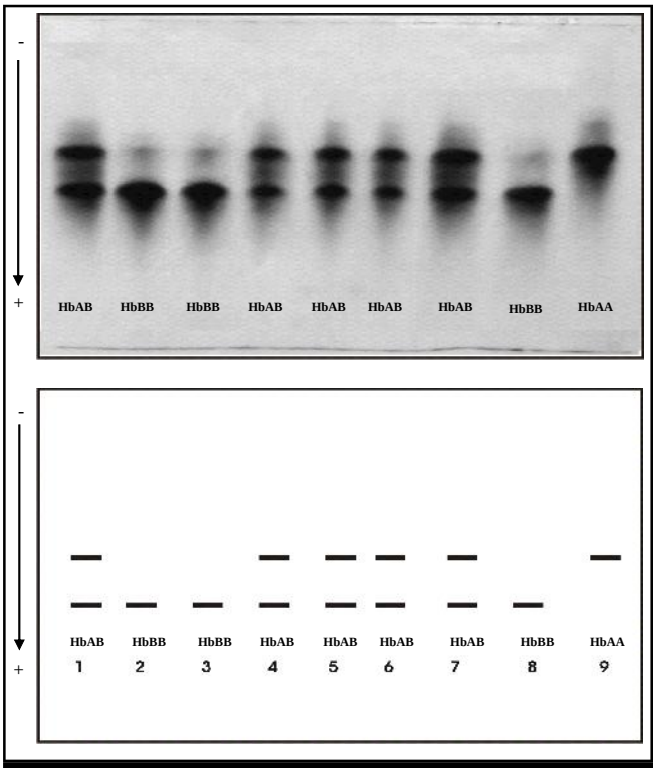

Fig. 1 : Electrophoretic pattern (PAGE) of haemoglobin in Gir crossbred cattle

The gene frequencies of Hb^B alleles was maximum in IFG (0.4563) and minimum in IFJG (0.4262) group of animals (Table 1). These results were supported by Rao *et al.* (1981) who reported that, the introduction of Jersey blood significantly increased the frequency of Hb^B. This was further supported by the higher frequency of Hb^B in Jersey and comparatively low frequency in Holstein Friesian among European cattle

Table 1 : Gene frequencies of haemoglobin phenotypes in different genetic groups and total Gir crossbred population

Hb types	Holstein Friesian (50%) + Gir (50%) (IFG)	Holstein Friesian (50%) + Jersey (25%) + Gir (25%) (IFJG)	Total Gir Crossbreds
Hb^A	0.5437 ± 0.044	0.5738 ± 0.0057	0.5567 ± 0.0220
Hb^b	0.4563 ± 0.044	0.4262 ± 0.0057	0.4433 ± 0.0033

Table 2 : Distribution of haemoglobin phenotypes in different genetic groups and total Gir crossbred population

Genetic group	No. of animals	Phenotypes						χ^2 (df = 2)
		Hb AA		Hb AB		Hb BB		
		Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
IFG	80	24	23.65	39	39.69	17	16.66	0.241^{NS}
IFJG	60	20	20.80	30	29.84	11	11.08	0.012^{NS}
Total gir crossbreds	141	44	43.68	69	69.61	28	27.71	0.010^{NS}

NS = Non significant ($\rho > 0.05$)

(Kirmani *et al.,* 1993; Senapati *et al.,* 1997).

The distribution of haemoglobin phenotypes (Table 2) showed the high incidence of phenotype Hb AB in all genetic groups i.e IFG and IFJG as well as total Gir crossbreds followed by Hb AA and Hb BB phenotypes (Table 2). The higher frequency of Hb AB phenotype was distinguished in their genetic groups, which possibly point to their adoption towards same environmental factor. Similar findings have been reported by Singh and Bhagi (1981) in three Grey cattle breeds. The expected phenotypes with various haemoglobin variants were calculated using gene frequencies obtained (Table 2). The chi-square test revealed that the observed number of phenotypic frequencies did not differ from expected frequencies indicating that all the genetic group and overall Gir crossbreed population under study were in Hardy-Weinberg equilibrium for haemoglobin variants. The genetic equilibrium exhibited by the Gir crossbred population revealed systematic pattern of deviation thus, indicating that the observed and expected values showed good agreements and their populations were random-bred with respect to this locus and did not differ significantly for Hb types distribution. These obtained results were well comparable with the results reported by Naik *et al.,* 1969; Shrimal and Parekh, 1982 and Kirmani *et al.,* 1993 in crossbred cattle populations.

The electrophoretic pattern of samples collected at different ages of an animal did not exhibit any change in the pattern / mobility of bands. Similar results were reported by Kirmani *et al.* (1993) in Gir crossbreds.

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