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Research Article

BLOOD PROTEIN TRANSFERRIN POLYMOROPHISM IN BLACK BENGAL GOAT

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ABSTRACT: The present investigation was carried out with an aim to explore the polymorphism of a blood protein tranferrin using starch gel electrophoresis technique in a total of unrelated 199 Black Bengal goats available in four different districts of West Bengal, India. Banding patterns of transferrin in starch gel revealed six phenovariants TfAA, TfAB, TfBC, TfBB, TfAC and TfCC comprising of three allelomorphs, Tf^A, Tf^B and Tf^C. The genotype frequencies were found to be observed 0.211, 0.347, 0.136, 0.106, 0.136 and 0.065 for six genotypes and the allelic frequencies were 0.452, 0.347 and 0.201 for three alleles, respectively. Result of Chi-square test revealed that the population under study was in Hardy Weinberg Equilibrium. There were polymorphism in Transferrin protein and the presence of differences among the frequencies of the three alleles by categories could be a source of genetic variation in Black Bengal goat.

Key words: Polymorphism, Transferrin, Protein, Black Bengal Goat.

INTRODUCTION

The Black Bengal goat is a precious germplasm for high prolificacy and fertility, superior quality of meat and skin, and adaptability to hot humid conditions in West Bengal along with adjoining part of the Jharkhand, Orissa, Bihar, Tripura and Assam states of India and Bangladesh (Moniruzzaman *et al.* 2002, Zeshmarani *et al.* 2007, Haldar *et al.* 2014). Although, ample of research works have already been conducted on different performance traits for improvement through conventional technique of selection and mating systems but genetic progress is time-consuming. Genetic improvement through biochemical polymorphism of transferrin, a heterogenous polymorphic blood protein belonging to the group of beta-globulins, participates in iron metabolism and in immune responses can give an impetus for marker assisted selection of Black Bengal goat (Slavov *et al*. 2004). Keeping these aspects in view the present study was carried out to investigate the existence of transferrin polymorphism in Black Bengal goat.

MATERIALS AND METHODS

Experimental Animals

The present study was conducted on randomly selected 199 Black Bengal does in four districts of West Bengal *viz*., Birbhum, Hooghly, North 24 Parganas and Nadia. The blood samples were collected from door to door of the farmer's house from different villages. The goats were reared on loose house managemental system mostly by landless farmers with grazing alone and no concentrate was given to them.

Collection of Blood Samples

About 6-8 ml of fresh blood was collected from each animal aseptically by puncturing jugular vein with 18 gauge needle. Then the blood was taken in a test tube and kept in a slanting tray to collect serum. The test tubes were kept for about one hour at room temperature and allowed to clot. Then serum separated out which was then poured into a centrifuge tube which was transported to the laboratory and centrifuged at 3000 rpm for 15 minutes. The serum from the top of the centrifuge tube was collected in dry aseptic rubber cap glass vials and stored in the deep freeze until used for the examination of serum protein transferrin polymorphism.

Hydrolysis Starch Gel Electrophoresis

The starch gel electrophoresis was done using the method as described by Smithies (1955) with minor modification to type the serum transferrin (Tf). The banding patterns were shown at 10% starch gel at 150 volt across the gel with 15 mA current. The staining and de-staining was done using the procedure as described by Smithies (1955). Destained gels were converted into gel transparencies by boiling the gels in a solution of 1% glycerol and 7% of glacial acetic acid for 5 minutes and the photographs were taken on the transilluminator. The polymorphic variants of transferrin (Tf) were recorded

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and analyzed. Identification of phenovariants of transferrin in the present study was done on the basis of number of bands on the gel.

Statistical Analysis

The parameters obtained for statistical analysis were the genotype categories, the conformity criterion (χ^2 test), the observed and expected phenotypes and the gene frequency of the alleles that corresponded to the transferring phenovariants. The frequency of genotypes and genes were computed by direct counting method for codominant loci (Falconer and Mackay 1996). Chi-Square (χ^2) test for goodness of fit was applied to find out whether the studied population was in Hardy-Weinberg Equilibrium or not with respect to transferrin phenovariants. The significance of calculated value was adjudged from the Table values of Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Transferrin Polymorphism

Research findings of genetic variability of Black Bengal goat for transferrin polymorphism based on banding pattern revealed that six different transferrin phenovariants *viz.*, TfAA, TfAB, TfAC, TfBB, TfBC, TfCC were identified in the whole studied population. Three phenovariants TfAA, TfBB and TfCC showed two bands each on starch gel while TfAB and TfBC phenovariants showed three bands each and Phenovariant TfAC was represented by four bands (Fig.1 to Fig. 3). In order of decreasing mobility towards anode, these six numbers of transferrin variants were found to be controlled by three co-dominant alleles, Tf^A, Tf^B and Tf^C of which Tf^A is the fastest and Tf^C is the slowest. It is eventually observed that all the six (6) transferrin phenovariants viz. TfAA, TfAB, TfAC, TfBB, TfBC and TfCC were prevalent in Black Bengal goat separately in each four districts of West Bengal under study.

Available literature suggested that the transferrin locus in goat is controlled at least by three co-dominant alleles Tf^A, Tf^B and Tf^C, of which Tf^A is the fastest and Tf^C is the slowest (Bhat 1986, Mandal *et al*. 1987, Shamsuddin *et al*. 1988) which is in accordance with the present findings. The homozygote phenotype possesses two bands, slower of which corresponds to the faster band of the next phenotype, if any. Heterozygote TfAB and TfBC were represented by three bands, due to overlapping of bands of two corresponding alleles, whereas TfAC shows four bands as the bands of A and C do not overlap. Trehan *et al*. (1981) in Alpine and Mandal *et al*. (1987) in Black Bengal also reported the similar finding. Therefore, it can be concluded that transferrin locus possesses at least three alleles and their relationship is co-dominant.

The present research findings is in accordance with Watanabe and Suzuki (1973) who also reported the existence of all six phenotypes in Korean, Philippine and Thai native goats by polyacrylamide gel electrophoresis. Similar observation was also reported by Kumar and Yadav (1988) in Jhakrana, Kutchi, Marwari and Sirohi breeds and Khanra (1998) in Black Bengal goat. While in contrary, Mandal *et al*. (1987) in Black Bengal goat and Erkoc *et al*. (1987) in Angora breed of goat recorded five phenotypes where they could not observed TfCC and TfAC respectively. Five co-dominant transferrin alleles viz. TfA, TfB, TfC, TfD and TfE were also observed in different breeds of sheep by Weimer *et al.* (1984), Iovenko (2002) and Slavov *et al.* (2004).

Genotype Frequency

The genotype frequencies of transferrin phenovariants irrespective of district and colour have been presented in Table 1 and the overall frequencies of TfAA, TfAB, TfAC, TfBB, TfBC and TfCC were found to be observed 0.211, 0.347, 0.136, 0.106, 0.136 and 0.065 respectively. The maximum number of animals showed TfAB phenotype followed by TfAA. The frequencies of the reminder genotypes were low to moderate and the least observed genotype was TfCC.

The data were also analyzed district wise (Table 1) to estimate the frequency of all six prevailing phenovariants. In Birbhum district it was observed that maximum number of Black Bengal goat showed TfAB phenotype and least observed phenotypes were TfCC. In Hooghly and Nadia districts the maximum number of animals showed TfAB

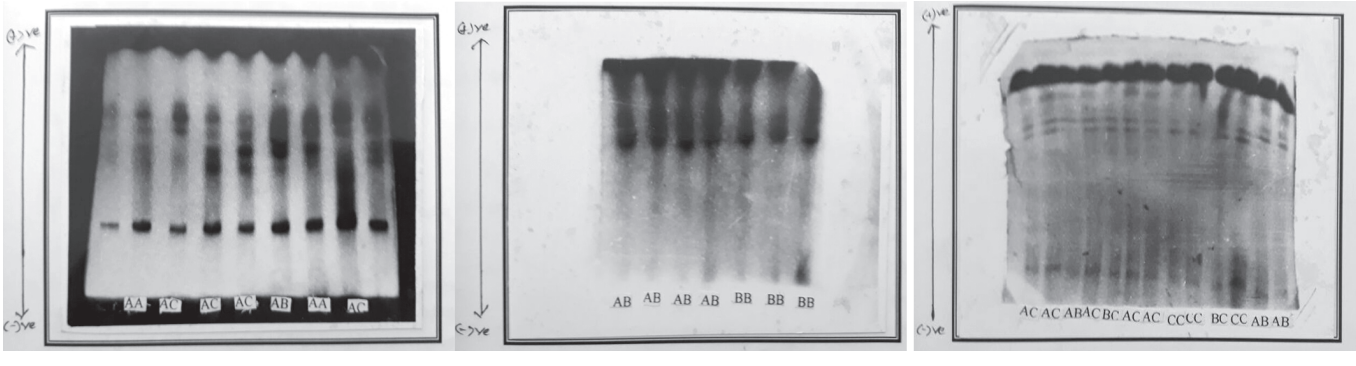

Fig. 1-3: Stained Starch Gel Showing Different Transferrin Phenovariants on the Basis of Number and Mobitily of Bands.

Table 1. Genotype and Gene Frequencies with respect to Transferrin in Black Bengal goat.

Phenotype	Genotype Frequency						Gene Frequency		
	TfAA	TfAB	TfAC	TfBB	TfBC	TfCC	Tf^A	Tf^B	Tf^C
					Birbhum District				
Black	0.188	0.313	0.188	0.000	0.188	0.125	0.438	0.251	0.313
Black and White	0.125	0.500	0.125	0.125	0.125	0.000	0.438	0.438	0.125
Brown	0.333	0.333	0.083	0.083	0.083	0.083	0.542	0.292	0.167
White	0.211	0.316	0.105	0.211	0.105	0.053	0.421	0.421	0.158
Total	0.218	0.345	0.127	0.109	0.127	0.073	0.455	0.345	0.200
Hooghly District									
Black	0.240	0.320	0.160	0.120	0.120	0.040	0.480	0.340	0.180
Black and White	0.000	0.000	0.000	0.500	0.500	0.000	0.000	0.750	0.250
Brown	0.222	0.333	0.111	0.111	0.222	0.000	0.444	0.389	0.167
White	0.200	0.400	0.000	0.200	0.200	0.000	0.400	0.500	0.100
Total	0.220	0.317	0.122	0.146	0.171	0.024	0.439	0.390	0.171
North 24 Parganas District									
Black	0.188	0.438	0.125	0.063	0.063	0.125	0.469	0.313	0.219
Black and White	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000
Brown	0.200	0.200	0.200	0.000	0.200	0.200	0.400	0.200	0.400
White	0.000	1.000	0.000	0.000	0.000	0.000	0.500	0.500	0.000
Total	0.174	0.391	0.130	0.043	0.087	0.174	0.435	0.283	0.282
Nadia District									
Black	0.239	0.283	0.174	0.087	0.174	0.043	0.467	0.315	0.217
Black and White	0.429	0.143	0.286	0.000	0.000	0.143	0.643	0.071	0.286
Brown	0.083	0.500	0.167	0.083	0.167	0.000	0.417	0.417	0.167
White	0.133	0.533	0.000	0.200	0.067	0.067	0.400	0.500	0.100
Total	0.213	0.350	0.150	0.100	0.138	0.050	0.463	0.344	0.194
Total Population									
Black	0.223	0.320	0.165	0.078	0.146	0.068	0.466	0.311	0.223
Black and White	0.222	0.278	0.167	0.111	0.111	0.111	0.444	0.306	0.250
Brown	0.211	0.368	0.132	0.079	0.158	0.053	0.461	0.342	0.197
White	0.175	0.425	0.050	0.200	0.100	0.050	0.413	0.463	0.125
Total	0.211	0.347	0.136	0.106	0.136	0.065	0.452	0.347	0.201

phenotype and the least observed phenotype was TfCC. In North 24 Parganas District it was found that maximum number of animals also showed TfAB phenotype with least observe phenotype in this district was TfBB. Hence, it is apparent that the TfAB phenovariant of transferrin protein was most prevalent in all four districts of West Bengal under study while TfCC was least prevalent in the entire study area except in North 24 Parganas where TfBB was in lowest frequency.

From the available literature it was also found that TfAB was in highest frequency in most of the reports with different breeds [Singh *et al*. (1977) in Barbari, Baruah and Bhat (1980) in Black Bengal, Trehan *et al*. (1981) in Alpine, Bhat *et al*. (1983) in Barbari and Jamnapari, Ugar *et al*. (1986) in Angora and Shamsuddin *et al*. (1988) in Malabar, Saanen x Malabar, Alpine x Malabar]. Highest frequency of TfAA was found in Beetal goat (Singh *et al*. 1977 and Trehan *et al*. 1981) and TfBB in Jamnapari goat as reported by Baruah and Bhat (1980) which were not in accordance with the present findings. The lowest frequency of TfCC transferrin phenovariant observed in this present study was in accordance with the findings of Khanra (1998) in Black Bengal goat. However, Baruah and Bhat (1980) observed the occurrence of TfAA to be lowest in Black Bengal goat which differs from the findings. Mandal *et al*. (1987) recorded lowest frequency for TfBB as he could not detect any TfCC.

Table 2. Observe and Expected number genotypes with respect to Transferrin protein in Black Bengal goats.

Phenotype	Frequency	AA	AB	AC	BB	BC	CC	χ^2 Value
				Birbhum District				
Black	Observed	3	5	3	0	3	2	2.469
	Expected	3.063	3.500	4.375	1.000	2.500	1.563	
Black and White	Observed	1	4	1	1	1	0	0.801
	Expected	1.531	3.063	0.875	1.531	0.875	0.125	
Brown	Observed	4	4	1	1	1	1	1.859
	Expected	3.521	3.792	2.167	1.021	1.167	0.333	
White	Observed	4	6	2	4	2	1	1.275
	Expected	3.369	6.737	2.528	3.368	2.526	0.474	
Total	Observed	12	19	7	6	7	4	2.769
	Expected	11.364	17.273	10.000	6.564	7.600	2.200	
Hooghly District								
Black	Observed	6	8	4	3	3	1	0.087
	Expected	5.76	8.160	4.320	2.890	3.060	0.810	
Black and White	Observed	0	0	0	1	1	0	0.222
	Expected	0.000	0.000	0.000	1.125	0.750	0.125	
Brown	Observed	2	3	1	1	2	0	1.056
	Expected	1.778	3.111	1.333	1.361	1.167	0.250	
White	Observed	1	2	0	1	1	0	1.050
	Expected	0.800	2.000	0.400	1.250	0.500	0.050	
Total	Observed	9	13	5	6	7	1	0.918
	Expected	7.902	14.049	6.146	6.244	5.463	1.195	
North 24 Parganas District								
Black	Observed	3	7	2	1	1	2	4.554
	Expected	3.516	4.688	3.281	1.5625	3.5156	0.7656	
Black and White	Observed	0	0	0	0	0	1	0.000
	Expected	0.000	0.000	0.000	0.000	0.000	1.000	
Brown	Observed	1	1	1	0	1	1	0.625
	Expected	0.800	0.800	1.600	0.200	0.800	0.800	
White	Observed	0	1	0	0	0	0	0.250
	Expected	0.250	0.500	0	0.25	0.250	0.000	
Total	Observed	4	9	3	1	2	4	6.946
	Expected	4.348	5.652	5.652	1.837	3.674	1.837	
Nadia District								
Black	Observed	11	13	8	4	8	2	0.848
	Expected	10.049	13.554	9.348	4.571	6.304	2.174	
Black and White	Observed	3	1	2	0	0	1	0.972
	Expected	2.893	0.643	2.571	0.036	0.286	0.571	
Brown	Observed	1	6	2	1	2	0	2.400
	Expected	2.083	4.167	1.667	2.083	1.667	0.333	
White	Observed	2	8	0	3	1	1	7.067
	Expected	2.400	6.000	1.200	3.750	1.500	0.150	
Total	Observed	17	28	12	8	11	4	1.205
	Expected	17.113	25.438	14.338	9.453	10.656	3.003	
Total Population								
Black	Observed	23	33	17	8	15	7	2.365
	Expected	22.369	29.825	21.437	9.942	14.291	5.136	
Black and White	Observed	4	5	3	2	2	2	1.254
	Expected	3.556	4.889	0.400	1.681	2.750	1.125	
Brown	Observed	8	14	5	3	6	2	1.671
	Expected	8.059	11.974	6.908	4.447	5.132	1.480	
White	Observed	7	17	2	8	4	2	4.444
	Expected	6.806	15.263	4.125	8.556	4.625	0.625	
Total	Observed	42	69	27	21	27	13	6.507
	Expected	40.704	62.412	36.181	23.925	27.739	8.040	

Gene Frequency

The resultant six transferrin phenovariants arises from different combinations of three alleles, viz. Tf^A, Tf^B and Tf^C in the Black Bengal goat population under study. The overall gene frequencies of Tf^A, Tf^B and Tf^C irrespective of district and colour were found to be 0.452, 0.347 and 0.201 for Tf^A, Tf^B and Tf^C, respectively (Table 1). The estimated frequencies of these three alleles in different districts under study were almost similar in pattern with the allelic frequencies in the whole population. The frequency of Tf^A allele ranged from 0.435 in North 24 Parganas District to 0.463 at Nadia District. The frequency of Tf^B allele ranged from 0.283 in North 24 Parganas District to 0.390 at Hooghly District. The frequency of Tf^C allele ranged from 0.171 in Hooghly District to 0.282 at North 24 Parganas District. The meager range Tf^A allele revealed its more adaptability in all four districts under study.

The estimated highest frequency of Tf^A as observed in the present study was in agreement with the findings of Trehan *et al*. (1981) in Alpine, Yaman (1982) in Angora, Bhat (1987) in Chegu and Chongthangi, Mandal *et al*. (1987) in Black Bengal, Wussow and Plischke (1990) in dwarf goats and Khanra (1998) in Black Bengal goat. Higher frequency of TfA allele was observed in many exotic sheep breeds as reported by Weimer *et al*. (1984) in Corriedale and Romney Marsh sheep and Iovenko (2002) in different sheep breeds of Ukraine. However, in contrary the higher frequency of Tf^B was reported by Watanabe and Suzuki (1973) in Thai Native goats, Singh et al. (1977) in Barbari and Beetal, Baruah and Bhat (1980) in Jamunapari, Barbari and Black Bengal, Trehan *et al*. (1981) in Beetal goat, Bhat *et al*. (1983) in Jamunapari and Barbari, Bhat (1986) in Jamunapari goat. The presence of Tf^C allele in lowest frequency in this present investigation was at per the findings of Watanabe and Suzuki (1973) in Thai Native goats, Trehan *et al.* (1981) in Alpine goat, Yaman (1982) in Angora Goat, Bhat *et al.* (1986) in Jamunapari, Bhat (1987) in Chegu and Changthangi goat, Mandal *et al.* (1987) in Black Bengal goat. It is obvious that absence and/or lowest frequency of the Tf^C allele in different goat breeds/ population as reported by various researchers and in this present research finding might be due to their poor adaptability in different region.

Hardy-Weinberg Equilibrium

In order to examine the estimated gene and genotypic frequencies with respect to Hardy-Weinberg equilibrium, the expected genotypic frequencies were compared with observed value under each district as well as in total population. The results have been tabulated in the Table 2. The research results revealed that the estimated χ^2 value was found to be less than the table value ($p<0.01$, 0.05) which indicated non-significant deviation between observed and expected phenotype/genotype in different districts as well as in whole population. Hence, the population under study was in Hardy-Weinberg equilibrium.

The above results were similar with the findings of Salerno *et al*. (1977) who reported that the population was in equilibrium in respect to transferrin in Toggenberg goat. Trehan *et al*. (1981) who also reported about the equilibrium population in respect to transferrin in crossbred and Vankan and Bell (1992) also found the population was in Hardy-Weinberg equilibrium in Australian goats. On the other hand, the significant difference between observed and expected genotype frequencies had been reported by Singh *et al*. (1977) in goat populations, Mandal (1987) in Black Bengal and Khanra (1998) in Black Bengal goats. Slavov *et al.* (2004) was also reported that according to the transferrin system, the 264 Ile de France sheep populations were not in genetic equilibrium due to the large variation in number between the observed and expected genotypes. The result of the equilibrium study revealed that the Black Bengal goat is maintaining equilibrium in respect to transferrin polymorphic locus in village level which gives an indication that random mating in a large population is being maintained at village level of West Bengal.

CONCLUSION

The findings of this present study to the results of other studies in literature have suggested that the considerable polymorphism of blood protein transferrin is still conserved in the population. In particular, the variation between the preliminary estimates seems to be at broad level in turn may considerably contribute to the indirect selection criteria. By using of these variations, it may be possible to increase the performance of Black Bengal goats at satisfactory level. Even so, in order to obtain a more definite conclusion further detailed works in this aspect is required on Black Bengal goat in their whole native tract.

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