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GENETIC DIVERSITY ANALYSIS OF CLASS II MHC IN INDIGENOUS POULTRY- A COMPARISON WITH COMMERCIAL BREED

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ABSTRACT

In poultry, Major Histocompatibility Compatibility Complex (MHC) plays a vital role in mounting immunogenic response to various antigens. The present investigation was undertaken as a pilot study to evaluate the polymorphism of the native Tellicherry breed against the commercial broilers at the MHC B-Lβ2 region by allele-specific PCR providing implications on genetic diversity for the design of efficient programmes for its characterization, conservation and upgradation. The extent of genetic variability and forces of selection were analyzed by Chi-square test, Ewens-Watterson's test for neutrality, the mean as well as the effective number of alleles per locus and Nei's expected heterozygosity. Population genetic analyses were carried out using the popgene (version 1.32) software. Ewens-Watterson test revealed natural selection to be favouring heterozygotes and acting against homozygotes and so the current population was more heterogeneous and had sufficient raw material for selection and improvement. Reinforcing this result, heterozygosity (0.8667) was found to be high in Tellicherry indicating its potential for genetic improvement.

Keywords: Diversity, Heterozygosity, MHC, Commercial broiler, Tellicherry

INTRODUCTION

In poultry, the classical Major Histocompatibility complex (MHC) evolved through natural selection showed a strong association with disease resistance and susceptibility (Hess and Edwards, 2002). The MHC is found in vertebrates encoding immunoglobulin like receptors which bind foreign peptides for presentation to T-cell (Kaufman, 2022). The

MHC known as the centre of the immune universe (Trowsdale, 1995), exist as highly polymorphic multigenic family, found to be conserved throughout the jawed vertebrate family. It exhibits high polymorphism at the antigen-binding site, which intrigues a key understanding of the genetic basis of pathogenic resistance (Hess and Edwards, 2002). A vast knowledge of the role of classical antigen presentation, combating various infectious conditions accumulated in past years made chickens the best model organism for immunogenetic studies (da Silva and Galllardo, 2020). Genomic analysis indicated that this essential element of the immune system that emerged in the course of the evolutionary history of terrestrial vertebrates had a significant role in an organism's existence and survival. High diversity at this locus indicates persistent ability to combat wide variety of pathogens (Matzaraki *et al*., 2017). In chickens, MHC is found on chromosome 16 encoded primarily by three tightly connected regions known as BF (class I), BL (class II), and BG (class IV) (Kaufman, 1999).

Tellicherry is the only indigenous poultry breed of Kerala, with its unique adaptive qualities to the tropical climate. They inhabit Western Ghats as well as coastal Kerala, as free-range feeding birds on backyard remnants. Their plumage is black with a gleaming bluish tinge on the hackle and back feathers (Vij *et al*., 2008). In recent years these native chicken are gaining importance due to the increase in demand for the special flavor of its dark hue meat. The alleged traditional medicinal quality of its meat further adds to its demand. They have undergone population decline and range fragmentation. Population bottlenecks are predicted to result in loss of variability leading to reduced disease resistance and adaptability (Orsted *et al*., 2019). So it became a subject of great scientific interest to find out whether sufficient variability still persist in this breed or not. Hence the present investigation was aimed to evaluate the status of diversity at antigen binding site of MHC B-Lβ2 region against broilers using allele specific - polymerase chain reaction technique.

MATERIALS AND METHODS

Whole blood up to 0.5 ml was collected individually from 30 each Tellicherry as well as commercial broiler chicken breeds by brachial venipuncture into 2 ml syringe and transferred to Ethylene Diamine Tetra Acetic acid (EDTA) coated vials (1 mg/ml of blood). Samples collected were stored at -20 °C until processing. Tellicherry birds were chosen from one of the native breeding tract in Kasaragod district of Kerala, India and those of broiler were collected from a private farm from the

same locality. By using phenol-chloroform extraction process Sambrook and Russel (1999), genomic DNA was extracted from the complete blood samples, resulting in relatively pure DNA preparation. Horizontal submarine agarose gel electrophoresis was performed to check the quality of genomic DNA using 0.8 per cent agarose gels. The purity of genomic DNA was checked using UV-Spectrophotometry. Only the genomic DNA samples lying in the ranges of OD ratio (260:280) between 1.7 to 1.9 were considered good and were used for further study. A pair of primers derived from relatively conserved segments of exon 2 of the B-Lβ II family genes coding antigen binding domain designed by Zheng *et al*. (1999) was used for initial amplification (Table 1). It was performed on all of the genomic DNA samples in order to get an amplicon sized 235 bp spanning from last two nucleotides of intron 1 to codon 71-78 of exon 2. 1 X PCR buffer, 4 mM MgCl2, 200 mM dNTPs, 20 p mole each of B-Lβ family-specific primers (Zheng *et al*., 1999), 1U Taq DNA polymerase and 100 ng of template DNA were used in the PCR mixture. An initial denaturation at 94 °C for 10 minutes was followed by 35 cycles of 94 °C for 30 seconds annealing temperature of 57 °C for 30 seconds, and an extension of 72 °C for 30 seconds. The quality of the PCR product obtained was checked in 1.5 per cent agarose gel containing ethidium bromide along with 100 bp ladder in a horizontal gel electrophoresis unit for the required band size (235 bp). The amplified product was visualized under UV and documented in gel-doc.

The B-Lβ family-specific PCR products were diluted 1: 100 times with distilled water and allele specific PCR was performed on 1 µl of the diluted products (Table 2). In each reaction volume 1XPCR buffer, 1.5mM $MgCl_2$, 200M each of dNTP, 20 pmol of each sequence specific primer, and 1U Taq DNA polymerase were used. The sequence-specific primers used by Zheng *et al*. (1999) were used to amplify particular alleles B_2, B_{13}, B_{15}, B_{19}, and B_{21} from the template PCR product following annealing temperatures of 55, 60, 50, 55, 55 degree Celsius, respectively. Remaining conditions were same as that mentioned in B-Lβ family-specific PCR. All the products of initial amplification were subjected to amplification with each allele specific primers (Table 1). Population genetic analyses were carried out using Popgene (version 1.32) software.

RESULTS AND DISCUSSION

The product of initial amplification was visualized as 235 bp band on 1.5 per cent agarose gel. On amplification and visualization (Plate 1.) with allele specific primers the genotype of each sample

Table 1 Sequences of primers used for AS-PCR analysis of exon 2 of MHC B-Lβ II family genes

B-Lβ II F	5'-CG TTC TTC TTC TRC GGT RBG AT -3'
B-Lβ II R	5'-TA GTT GTG CCG GCA GAM CSY G -3'
B_2 F	5' – TTC TTC TAC GGT GTG ATA TT – 3'
B_2 R	5' – CCG GCA GGG CCC GTC CGC TAT -3'
B_{13} F	5' – ATC TAC AAC CGG CAG CAG TTA – 3'
B_{13} R	5' – CGT GTC CAC CTC ATT CCT TTT TA – 3'
B_{15} F	5'- TTC TTC CAC GGT GTG ATA GC -3'
B_{15} R	5' – CCG GCA GGG CCC GTC CAC TG – 3
B_{19} F	5'- TTC TTC TGC GGT GCG ATA TCC - 3'
B_{19} R	5' – CCT GTC CAC TTC ATT CAT TC – 3'
B_{21} F	5'- TTC TTC TAC GGT AAG ATA GG – 3'
B_{21} R	5' – CCT GTC CAC TTC ATT CAT TA -3'
(R=A or G, M=A or C, S=G or C, Y = C or T, B=G, C or T)	

(Table 2) could be clearly identified. The genotype $B_{13}B_{15}$ was predominant in Tellicherry whereas $B_{13}B_{13}$ and $B_{15}B_{15}$ genotypes were prominent in commercial broiler suggestive of a more heterozygous or diverse nature of Tellicherry population. In Tellicherry genotypes $B_{13}B_{19}$, $B_{15}B_{19}$, B_2B_{19} and B_2B_2 were found with the lowest frequency. Among the commercial broiler population $B_{13}B_{15}$, $B_{15}B_{19}$, $B_{15}B_{21}$, B_2B_{13} and B_2B_{15} were observed with the lowest frequency. Compared to these populations B_2B_{13} showed highest genotypic frequency and the lowest was reported by $B_{19}B_{19}$ genotype in Kadaknath population (Thakur *et al*, 2017). Shanaz *et al*. (2005) reported highest frequency of $B_{15}B_{19}$ genotype in bantamised White Leghorn and $B_{19}B_{19}$ in Bantam. A higher frequency of B_2B_{13} and lowest of $B_{15}B_{19}$ were observed in Jabalpur dual coloured type birds (Jha *et al*., 2017). In Caribro-Vishal chickens $B_{15}B_{19}$ genotype was found predominant whereas $B_{13}B_{19}$ and $B_{15}B_{21}$ were least (Thakur *et al*., 2016). Differences in genotypic frequencies of different breeds were due to varying degrees of different evolutionary forces and environmental effects (Thakur *et al*, 2017).

B_{13} haplotype showed the highest allelic frequency in Tellicherry and commercial broiler populations (Table 3). The lowest frequency was exhibited by the B_{19} allele in the Tellicherry breed and the B_2 allele in the commercial broiler chicken. The B_{21} (0.2) haplotype was present in the commercial broiler population whereas

Table 2 Genotypic frequencies and chi-square values of Tellicherry and Broiler populations

Sl No.	Broiler			Tellicherry		
	Genotypes	Freq	Exp freq	Genotypes	Freq	Exp freq
1	B13B13	0.17	0.08	B13B13	0.10	0.16
2	B13B15	0.03	0.13	B13B15	0.43	0.25
3	B13B19	0.06	0.11	B13B19	0.03	0.04
4	B13B21	0.10	0.11	B15B19	0.03	0.03
5	B15B15	0.17	0.05	B2B13	0.13	0.17
6	B15B19	0.03	0.09	B2B15	0.20	0.14
7	B15B21	0.03	0.09	B2B19	0.03	0.02
8	B19B19	0.10	0.04	B2B2	0.03	0.47
9	B21B21	0.13	0.04			
10	B2B13	0.03	0.05			
11	B2B15	0.03	0.04			
12	B2B19	0.10	0.03			
13	chi-square		28.29*	chi-square		5.99^{NS}

*significant
Chi-square values given are calculated

Table 3 Allelic frequencies of Tellicherry and Broiler populations

Sl No.	Alleles	Broiler	Tellicherry
1.	B_2	0.08	0.22
2.	B_{13}	0.28	0.4
3.	B_{15}	0.23	0.32
4.	B_{19}	0.2	0.05
5.	B_{21}	0.2	0

in Tellicherry chicken it was absent. In Kadaknath and Bantam populations (Thakur *et al*., 2016; Shanaz *et al*., 2005) B_{21} allele was absent whereas in White Leghorn it was found in lower frequency (Jha *et al.*, 2017). In Caribro-Vishal chicken B_{19} and B_{15} alleles were predominant (Thakur *et al*., 2016). In the Tellicherry population frequency of B_2 homozygote was 0.03. The difference in allelic pattern among populations reflected the effect of environment, geographic variation and breeding policies.

Chi-square values of Tellicherry (5.99, df=7) was found to be insignificant whereas that of Broiler (28.28, df=11) was highly significant (Table 2). So the Tellicherry population was in Hardy-Weinberg equilibrium with respect to the MHC B-Lβ loci. Random mating over generations has resulted in an equilibrium state with respect to the MHC genotypes in the Tellicherry breed. A similar result was obtained in the Kadaknath population where non-significant Chi-square values indicated it to be under Hardy-Weinberg

Table 4 Ewens- Watterson test for neutrality and effective number of alleles

Population	Observed F value	Expected F value	L95	U95	SE	Observed no. of alleles	Effective no. of alleles
Tellicherry	0.3206	0.5325	0.2994	0.8728	0.0246	4	3.1196
Broiler	0.2217	0.4462	0.2528	0.7844	0.0195	5	4.5113

Table 5 Nei's expected and observed heterozygosity as well as homozygosity

Population	Nei's expected heterozygosity	Expected heterozygosity	Observed heterozygosity	Expected homozygosity	Observed homozygosity
Tellicherry	0.6794	0.6910	0.8667	0.3090	0.133
Broiler	0.7783	0.7915	0.4330	0.2085	0.5667

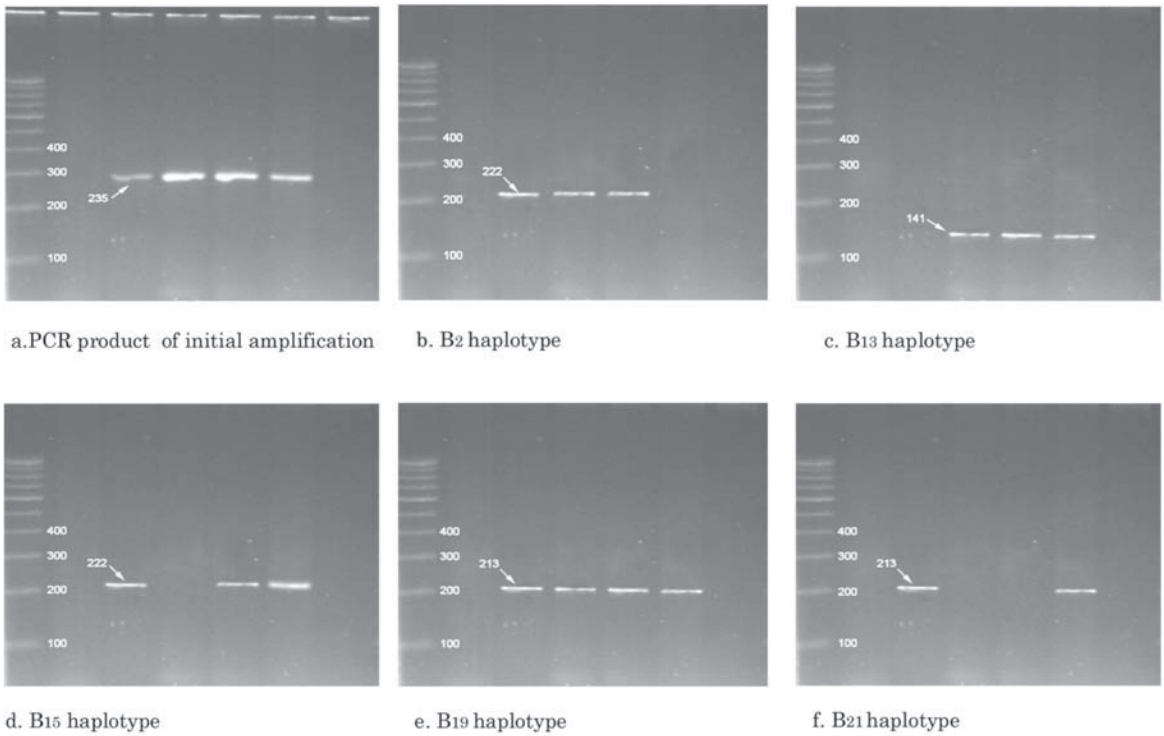
Table 6 Nei's measures of genetic identity and genetic distance between Tellicherry and Broiler populations.

Parameter	Nei's original measure	Nei's unbiased measure
Genetic identity	0.8222	0.8421
Genetic distance	0.1958	0.1718

equilibrium (Thakur *et al.*, 2017). Perhaps it further indicated a lack of selective advantage for particular MHC alleles, resulting in balanced reproductive and survival rates (Crow and Kimura, 1970).

Ewens-Watterson's test was done to findout evidence of deviation from neutral expectation in selection. The observed F-value in Tellicherry stayed within the standard range of U95 (0.8728) and L95 (0.2994). Expected F-value values of Tellicherry (0.5325) was higher in comparison with the observed values (0.3206) revealing natural selection to be working against homozygotes. So the heterozygotes were favoured (Table 4). Present analysis revealed that the F value of Tellicherry MHC loci did not deviate from neutral expectation, indicating presence of diversifying selection. Similar results were reported in the Red jungle fowl population (Worley *et al*., 2008), where none of the MHC loci deviated from neutrality proving the presence of diversifying selection.

The mean observed number of alleles and effective number of alleles were found to be 4 and 3.1196, respectively in Tellicherry, whereas it was found to be 5 and 4.5113 respectively in commercial broiler. The effective number of alleles in a population is an indicator of the extent of genetic variation (Table 4). In the current study, the effective numbers of alleles in both populations were lesser than the



a.PCR product of initial amplification b. B_2 haplotype c. B_{13} haplotype

d. B_{15} haplotype e. B_{19} haplotype f. B_{21} haplotype

Plate 1. a. amplified product of initial amplification with MHC B-Lβ II family specific primers b. to f. images of AS-PCR products for five haplotypes along with 100 bp ladder (sizes of bands given in base pairs)

observed number of alleles indicating genetic drift and mutation acting on alleles causing divergence of frequencies among subpopulations.

High heterozygosity indicated splendid genetic variability and low heterozygosity means sparse of it. If the observed heterozygosity (0.4330) is lower than the expected (0.7915) as in the commercial broiler, it might be attributed to the discrepancy to forces such as inbreeding, dominance etc. If heterozygosity (0.8667) is higher than expected (0.6794) as in Tellicherry, an isolate-breaking effect shall be suspected (the mixing of two previously isolated populations) (Table 5). The broiler has been under intensive selection and breeding for economic traits for several generations showed lower heterozygosity at MHC loci. Sri Lankan Karuwalagsewa, Nigerian, Bangladeshi, Tanzanian Chunya as well as Msimbazi and Indian Kadaknath as well as Ghagus had higher or similar MHC heterozygosity (Thakur *et al*., 2017; Manjula *et al*., 2021; Mwambene *et al*., 2019; Haunshi *et al*., 2020). At the same time Dahlem red, Nicobari and White Leghorn populations had low heterozygosity compared to Tellicherry (Thakur *et al*., 2017; Haunshi *et al*., 2020). In addition to the potential for improvement, heterozygotes have a better chance for adaptation and survival under

harsh climatic conditions. As expected, observed homozygosity yielded a lower value (0.133) in Tellicherry compared to broiler (0.5667).

The fraction of identical genes in two groups is known as genetic identity. Nei's original measure of genetic identity between Tellicherry and broiler were found to be 0.8222 and Nei's unbiased measure of genetic identity to be 0.8421. Compared to the present result very low genetic identity was reported between White Leghorn and KNC white of Korea as well as Ghagus and Nicobari (Thakur *et al*., 2017; Haunshi *et al*., 2020; Manjula *et al*., 2021). The Nei's original measure of genetic distance between Tellicherry and broiler was estimated to be 0.1958 and Nei's unbiased measure of genetic distance to be 0.1718. Compared to this result very high genetic distance was reported between White Leghorn and KNC white of Korea as well as Ghagus and Nicobari (Thakur *et al*., 2017; Haunshi *et al*., 2020; Manjula *et al*., 2021). At the same time based on polymorphism analysis with SNP chip data close relationship with Korean KNC with Rhode Island Red and Cornish was revealed (Manjula *et al*., 2021).

CONCLUSION

In Tellicherry $B_{13}B_{15}$ heterozygote was prominent whereas in commercial broiler $B_{13}B_{13}$ and $B_{15}B_{15}$ homozygotes were predominant. This result indicated more heterozygous nature of Tellicherry breed compared to broiler. In both the populations B_{13} haplotype was observed to be showing highest allelic frequency. Insignificant Chi-square values indicated Tellicherry population to be in Hardy-Weinberg equilibrium with respect to the MHC B-Lβ loci whereas that of broiler was not. Ewens-Watterson's test revealed that natural selection acting on B-Lβ II locus was favouring heterozygotes and acting against homozygotes thus rendering the resultant population more heterozygous and diverse. At the same time, lesser effective number of alleles than observed value indicated genetic drift and mutation acting on the population leading to divergence of allelic frequencies. In Tellicherry chicken, observed heterozygosity was found to be much higher when compared to broilers. The heterozygotes were reported to be better in adaptation and survival under harsh climatic condition. Though the population size of Tellicherry population has declined, the genetic variation at antigen binding site of class II MHC has not reduced significantly indicating persistent ability to combat wide variety of pathogens. The high variability in Tellicherry implies that this breed is suitable for improvement in production performance by application of adequate selection and breeding methods.

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