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Study of Polymorphism at MHC B-Lβ (Class II) Family Alleles Using PCR-SSP in Naked Neck Chickens

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Abstract

The Major Histocompatibility Complex (MHC) plays a crucial role in disease resistance and immune response in poultry. This study investigated the polymorphism at MHC B-Lβ (class II) family alleles in Naked Neck chickens using the Polymerase Chain Reaction-Sequence Specific Primers (PCR-SSP) technique. Sixty Naked Neck chickens were analyzed for five standard haplotypes (B_2, B_{13}, B_{15}, B_{19}, and B_{21}). The results revealed significant genetic diversity with 10 distinct genotypes identified. Allele B_{19} showed the highest frequency (0.392), followed by B_{15} (0.258), B_2 (0.183), B_{21} (0.125), and B_{13} (0.042). The population showed Hardy-Weinberg disequilibrium ($\chi^2 = 36.179$, $P<0.05$), indicating non-random mating or selection pressure. These findings provide valuable insights into the genetic structure of Naked Neck chickens and their potential for disease resistance breeding programs.

Introduction

The poultry industry faces significant challenges from infectious diseases, particularly coccidiosis, which causes substantial economic losses worldwide. In India alone, coccidiosis-related losses were estimated at Rs 1.14 billion in 2003-04. Traditional disease control methods rely heavily on chemotherapeutics, but the emergence of drug-resistant pathogens and restrictions on antimicrobial use necessitate alternative approaches. Genetic resistance offers a sustainable solution for improving disease resistance in poultry. The Major Histocompatibility Complex (MHC), initially known as the 'B Complex' in chickens, represents one of the most extensively studied gene systems associated with immune response and disease resistance. The MHC is a polymorphic, multigene system whose products play a primary role in antigen presentation to T lymphocytes during humoral and cell-mediated immune responses. Naked Neck chickens, characterized by their distinctive featherless neck region, represent an important genetic resource with potential advantages in tropical climates. However, limited information exists regarding MHC polymorphism in this breed. This study aims to characterize the genetic diversity at MHC B-Lβ (class II) family alleles in Naked Neck chickens using PCR-SSP methodology, providing foundational data for future breeding programs focused on disease resistance.

Materials and Methods

Experimental Birds

Sixty-day-old Naked Neck chicks were obtained from the Central Avian Research Institute, Izatnagar, Uttar Pradesh. Fertile eggs were hatched at the Department of Poultry Science, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur.

Housing and Management

Birds were maintained in brooder batteries under controlled conditions with continuous lighting. Ambient temperature was gradually decreased from 32°C on day 1 to 20°C at the experiment's end. Standard chick diet free of anti-coccidial additives and sanitized tap water were provided ad libitum. Strict coccidian-free conditions were maintained throughout the experimental period.

Blood Collection and DNA Isolation

Blood samples (1 ml) were collected from the wing vein of each bird using EDTA vacutainers. Genomic DNA was extracted using the method described by John *et al*. (1991) with minor modifications. The protocol involved:

- Blood lysis using solution-I (10mM Tris, 10mM KCl, 10mM $MgCl_2$, pH 7.6)
- Nuclear lysis with solution-2 containing SDS
- Phenol-chloroform extraction
- DNA precipitation with sodium acetate and ethanol
- Final resuspension in 0.3X TE buffer

DNA Quality Assessment

DNA concentration and purity were assessed using UV spectrophotometry (NanoDrop 1000, Thermo Scientific).

Samples with $OD_{260}/_{280}$ ratios between 1.7-1.9 were considered acceptable. DNA quality was confirmed through 0.8% agarose gel electrophoresis, and samples were diluted to 30 ng/µl for PCR amplification.

PCR-SSP Analysis

Primary PCR Amplification

Initial amplification of MHC B-Lβ II family genes was performed using degenerate primers:

- Forward: 5'-CG TTC TTC TTC TRC GGT RBG AT-3'
- Reverse: 5'-TA GTT GTG CCG GCA GAM CSY G-3'

PCR conditions included initial denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation (94°C, 45s), annealing (54°C, 40s), and extension (72°C, 30s), with final extension at 72°C for 15 minutes.

Sequence-Specific PCR

Five haplotype-specific primer pairs were used for PCR-SSP targeting B_2, B_{13}, B_{15}, B_{19}, and B_{21} alleles. Diluted primary PCR products (1:10) were used as templates with specific annealing temperatures and cycle numbers optimized for each haplotype.

Statistical Analysis

Allelic and genotypic frequencies were calculated using PopGene 32 software. Hardy-Weinberg equilibrium was tested using chi-square analysis. Homogeneity of allele distribution was assessed across different genetic groups.

Results and Discussions

DNA Quality and PCR Amplification

All DNA samples showed high purity with $OD_{260}/_{280}$ ratios between 1.7-1.9. Primary PCR amplification yielded a 235 bp product in all samples, confirming successful amplification of MHC B-Lβ II family genes.

Allelic Frequency Distribution

Five alleles were detected in the Naked Neck population with varying frequencies:

Allele	Frequency
B_{19}	0.392
B_{15}	0.258
B_2	0.183
B_{21}	0.125
B_{13}	0.042

Allele B_{19} was most prevalent, accounting for nearly 40% of all alleles, while B_{13} showed the lowest frequency at 4.2%.

Genotypic Frequency Distribution

Ten distinct genotypes were identified in the Naked Neck population:

Genotype	Number of Birds	Frequency
$B_{15}B_{19}$	13	0.217
$B_{19}B_{19}$	13	0.217
B_2B_{19}	8	0.133
B_2B_{15}	6	0.100
B_2B_2	4	0.067
$B_{15}B_{21}$	4	0.067
$B_{21}B_{21}$	4	0.067
$B_{13}B_{21}$	3	0.050
$B_{15}B_{15}$	3	0.050
$B_{13}B_{15}$	2	0.033

The heterozygous genotype $B_{15}B_{19}$ and homozygous genotype $B_{19}B_{19}$ were equally prevalent, each representing 21.7% of the population.

Hardy-Weinberg Equilibrium Analysis

Chi-square analysis revealed significant deviation from Hardy-Weinberg equilibrium ($\chi^2 = 36.179$, $P<0.05$), indicating that the Naked Neck population was not in genetic equilibrium at the MHC B-Lβ locus.

Genetic Diversity

The immune competence of the host could be evaluated using certain immune related parameters such as the antibody production, lymphocyte proliferation, phagocyte activity, parasite load etc. (Bacon,1987, Bumstead *et al*., 1995 and Briles *et al*., 1977). These immune parameters describe the immune responsiveness that could be correlated with resistance or susceptibility to a pathogen. Yet, it is essential to discern the molecular basis for variation in the immune response and disease resistance. The main mediators in the communication of the immune cells are membrane proteins such as molecules of the major histocompatibility complex (MHC), T cell receptors (TcR) and immunoglobulins (B cell receptors), and secreted proteins such as cytokines and antibodies (Hawken *et al*., 1998). At the molecular level, a difference in immune competence could mainly be attributed to the efficacy of the immune mediator molecules and their diversity. Due to the complexity of the immune system, the actual functional mechanism of each immune mediator protein and its marker significance in disease resistance is obscure. In the chicken, the association between MHC polymorphism and resistance or susceptibility differences to infectious pathogens had long been recognized (Briles *et al*., 1977 and Lamount,1991).

In most reports, the MHC is outlined in association to resistance or susceptibility to specific agents such as the Marek's disease virus. Yet, the role of the other immune mediator proteins and their immunogenetics in disease resistance differences is less described in chickens (Lillehoj *et al*.,

2007), Chapman and Jeffers, 2024). The uniqueness of the MHC molecule in each individual makes the immune response to vary between the MHC haplotypes. Since the T cell response is restricted to MHC-bound antigenic fragments, the diversity of the MHC's antigen-binding region has crucial immunological consequences for the induction of the adaptive immune responses (Lamount,1991). In the present study, analysis for genetic aspects of immune response in particular the role of MHC (B-Lβ II genes), which is the best characterized family of host genes, modulating response to a variety of antigens and pathogenic challenges was conducted using PCR-SSP.

Population Genetic Structure

The significant deviation from Hardy-Weinberg equilibrium indicates non-random mating patterns or selection pressures acting on the MHC locus. This could result from: Selective breeding practices favoring certain MHC alleles, population bottlenecks or founder effects, non-random mating based on MHC genotypes and selection pressure from pathogen exposure. The differences in allelic frequencies observed in the present study might be because different populations of different breeds maintained under different sets of environmental conditions are subject to different evolutionary forces to varying degree. In addition, sampling fluctuations might also have contributed to the differences in allelic frequencies in different breeds and populations. Further, intermixing of populations from different geographical locations and hybridization accompanied by genetic difference might have also

contributed to this high degree of genetic diversity among breeds/lines.

The genetic equilibrium condition observed in some populations may be due to random mating for MHC genotypes over the generations. The other probable reasons for this may be non-selective advantages for the different MHC alleles over each other, different reproductive and survival rates of different genotypes and state of balance between different forces which change the gene frequencies. However, the genetic disequilibrium observed in Naked Neck population may be attributed to absence of factors responsible for maintenance of genetic equilibrium.

Implications for Disease Resistance

The presence of multiple MHC alleles in Naked Neck chickens provides a broad genetic foundation for immune response diversity. The high frequency of B_{19} allele may indicate its association with favorable immune traits in this population. The substantial presence of heterozygous genotypes (particularly $B_{15}B_{19}$) suggests potential heterozygote advantage in immune function.

PCR-SSP Methodology and Technical Considerations

A set of degenerating primers was used for carrying out first round of PCR amplification in the present study. Both upstream and downstream primers were degenerate at few positions to optimize amplification of all known chicken B-Lβ II genes family alleles. PCR-SSP technique is one of the variations of PCR based on the amplification of the refractory mutation system (ARMS) method

(Newton *et al*.,1989) using sequence specific primers developed by (Ollerup and Zetterquist, 1993) and Zheng *et al*.,1999) proposed the method as an adjunct to serological typing for B-haplotypes. Accordingly, PCR-SSP was not performed directly from chicken genomic DNA, but instead amplified B-LβII loci with B-LβII family primers as the first step. This primary PCR step served two purposes: one was to generate B-LβII family specific DNA templates for the secondary PCR-SSP; the other was to ensure that all the DNA samples and reaction conditions were appropriate for the PCR test.

The secondary PCR amplification i.e. sequence specific polymerase chain reaction for targeted haplotypes i.e. B_2, B_{13}, B_{15}, B_{19} and B_{21} yielded amplicons of 222 bp, 141 bp, 222 bp, 213 bp and 213 bp size, respectively in the present study. Similar findings pertaining to base pair size of the above haplotypes were also reported by Zheng *et al.* (1999) who also used the PCR-SSP method to type B-Lβ II family alleles in broiler chicken. They observed that haplotypes with identical B-Lβ II family sequences produced identical reaction patterns in PCR-SSP: B_{21}, BA_4 and BA_7 haplotypes reacted identically, despite differing in the B-G regions. In their study most of the B homozygotes yielded strong amplification products with only one primer pair with the exception of BA_8, BA_9 and BA_{12} haplotypes. Findings similar to the present one have also been reported by (Shanaz *et al.*, 2005) in Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) with regards to amplification pattern of standard haplotypes used in the present study. Understanding MHC polymorphism in Naked Neck chickens enables:

- Development of marker-assisted selection programs for disease resistance
- Maintenance of genetic diversity in breeding populations
- Strategic crossbreeding to optimize immune competence
- Conservation of valuable genetic resources

Comparative Analysis with Other Chicken Breeds

Compared to other chicken breeds studied using similar methodologies, Naked Neck chickens showed unique allelic distribution patterns. Similar study for MHC haplotype conducted on Bantam, Bantamised White leghorn (BWLH) and White Leghorn (WLH) was reported by Shanaz *et al.* 2005. In their study, different frequency for different haplotypes were reported. Allele B_{19} (0.680) was predominant followed by B_{15} (0.263) and B_2 (0.055) in Bantam, with B_{21} being absent in this genetic group. In Bantamised White leghorn (BWLH), B_{19} (0.486) and B_{15} (0.444) were observed predominantly followed by B_2 (0.065), whereas in WLH, B_{15} (0.402) had the highest frequency followed by B_{19} (0.333), B_2 (0.138) and B_{21} (0.125). Allele B_{21} was observed only in WLH at lower frequency. The relatively high frequency of B_{21} allele (12.5%) in Naked Neck chickens distinguishes this population from many commercial breeds where this allele is often absent or rare. In the comparative study by Shanaz *et al.* (2005), B_{21} allele was completely absent in Bantam chickens and present only in White Leghorn at 12.5% frequency, similar to our findings in Naked Neck chickens.

Regarding genotypic distribution, in Bantam genetic group, predominantly two genotypes, $B_{15}B_{19}$ and $B_{19}B_{19}$ were observed with respective frequencies of 0.416 and 0.472. Other two genotypes, B_2B_2 and $B_{15}B_{15}$ were observed at equal frequencies (0.055). In Bantamised WLH, five genotypes were observed, among them heterozygote $B_{15}B_{19}$ (0.832) was predominant while B_2B_2, $B_{19}B_{19}$ and B_2B_{19}, $B_{15}B_{15}$ were observed at low frequencies. In WLH, nine genotypes were observed, in that homozygous $B_{15}B_{15}$ (0.249) and $B_{19}B_{19}$ (0.194) were predominant followed by $B_{15}B_{19}$ (0.138), B_2B_{15}, B_2B_{19} and $B_{15}B_{21}$, equally at 0.082 and B_2B_2, $B_{19}B_{21}$ and $B_{21}B_{21}$ equally at 0.055.

Conclusions

This study successfully characterized MHC B-Lβ (class II) polymorphism in Naked Neck chickens using PCR-SSP methodology. The findings reveal significant genetic diversity with five alleles and ten genotypes, non-random distribution of MHC alleles indicating selection pressure, potential for utilizing this genetic diversity in disease resistance breeding and need for further investigation into functional significance of identified alleles. The genetic disequilibrium observed suggests ongoing evolutionary forces shaping the MHC diversity in this population. These results provide a foundation for developing breeding strategies that leverage MHC diversity to enhance disease resistance while maintaining the unique characteristics of Naked Neck chickens.

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