*Original Article. DOI: 10.22059/ijvm.2018.253776.1004885* *Iranian Journal of Veterinary Medicine*

# MHC IIB Genetic Diversity and its Association With Humoral Immune Responses in Commercial Turkey

**Hassan Al-Karagoly[1], Gholamreza Nikbakht Brujeni[1], Atefeh Esmailnejad[2], Mohammad Hassanzadeh[3], Tohid Tolouei[3]**

[1]*Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

[3]*Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

## Abstract:

**BACKGROUND:** Major histocompatibility complex (MHC) is one of the best characterized genetic regions controlling immune responses against vaccines. Identifying the association between MHC haplotypes and improved immune responses would be useful in genetic breeding strategies in animals.

**OBJECTIVES:** MHC class II B genetic diversity and its association with humoral immune responses against Newcastle vaccine (NDV) were evaluated in commercial turkey poults (meleagris gallopavo).

**METHODS:** A total of 92 turkey poults were vaccinated with live VG/GA strain of Newcastle disease vaccine at age of 10 and 20 days. Serum NDV specific IgY was assessed by indirect enzyme linked immunosorbent assay (ELISA) and MHC II B polymorphism was determined using high resolution melting curve (HRM) technique and DNA sequencing method. Effects of alleles on humoral immune responses were evaluated by multivariate regression analysis and GLM procedures.

**RESULTS:** A total of 8 HRM profiles and 10 alleles were identified in this population. B1-1*3, B1-1*4 and B1-1*5 alleles were significantly associated with lower antibody responses against vaccine in commercial turkey poults.

**CONCLUSIONS:** Three alleles reported in this study were associated with reduced immune responses against NDV vaccine in turkey population. Due to such negative associations, molecular breeding programs based on specific genetic markers should be implemented with great caution.

## Keywords:

Antibody, Immunity, MHC, Turkey, Vaccine

**Correspondence**

Gholamreza Nikbakht Brujeni, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Tel: +98(21) 61117057, Fax: +98(21) 66427517, Email: nikbakht@ut.ac.ir

Received: 5 February 2018

Accepted: 20 May 2018



**How to Cite This Article**

Alkaragoly, H., Nikbakhat Brujeni, G., Esmailnejad, A., Hasanzadeh, M., Tolouei, T. (2018). MHC IIB Genetic Diversity and its Association With Humoral Immune Responses in Commercial Turkey. Iran J Vet Med, 12(4), 347-356. doi: 10.22059/ijvm.2018.253776.1004885

## Introduction

Newcastle disease (ND) is a highly contagious viral disease that represents a considerable threat to the poultry industry all over the world. It is caused by Newcastle disease virus (NDV) which has been designated as serotype 1 of Avian Paramyxovirus (APMV-1) (Mishra et al., 2000). NDV is able to infect almost all avian species (domestic and wild species), of which chickens are the most susceptible. Dyspnea, gasping, cyanosis of comb, wattle, crop dilatation, catarrh, foamy mucus in the pharynx, ataxia, paralysis, and torticollis are the most obvious signs of Newcastle disease that affect the respiratory tract, gastrointestinal tract, circulatory and nervous systems (Senne et al., 2004).

Different avian species show different patterns of disease resistance or susceptibility to the Newcastle. Variations in the level of immune responses among different populations can be as a result of several factors including genetic background, environmental factors and virus strain (Alexander et al., 2012; Norup et al., 2011). Major histocompatibility complex (MHC) is one of the best characterized genetic regions controlling disease resistance and susceptibility in chicken. Chicken MHC can influence the immune responses against viral diseases, including Marek disease (Briles et al., 1977; Hunt and Dunn, 2013), Rous sarcoma tumor (Suzuki et al., 2010; Taylor Jr, 2004), Infectious bursal disease (Butter et al., 2013; Juul-Madsen et al., 2006), Newcastle disease (Norup et al., 2011), and Avian Influenza (Hunt et al., 2010). Therefore, MHC could be considered as a genetic marker for identifying immune responses and their association with resistance or susceptibility to diseases.

Our previous studies have shown that polymorphisms at chicken MHC region are associated with immune response to vaccination and productive traits (Emam et al., 2013; Nikbakht and Esmailnejad, 2015). The architecture of MHC region in turkey is similar to chicken. It is divided into two genetically unlinked regions designated as MHC-B and MHC-Y loci located on the same microchromosome (Chaves et al., 2010). The MHC-B locus (BF–BL region) is generally considered as the homolog to the mammalian MHC, which contains most of the antigen processing and presenting genes. MHC-Y locus contains non-classical MHC genes, lectin-like loci, and other unknown genes with varied effects on disease susceptibility (Chaves et al., 2010). Association studies in chicken typically concentrated on the peptide-binding region (PBR) encoded in exons 2 and 3 of class I and exon 2 of the class IIB genes.

In this study, MHC genetic diversity was evaluated in commercial turkey poults (meleagris gallopavo), vaccinated by lentogenic VG/GA Newcastle vaccine. Subsequently, association of MHC haplotypes with humoral immune responses (IgY level) was also analyzed. Although the efficacy of VG/GA vaccine has been evaluated and proved in commercial broiler chickens, there is no information regarding the MHC genotypes and their association with antibody response to this vaccine in turkey.

## Material and Methods

**Turkey poults and Housing:** A total of 92 one day old male commercial turkey poults (Meleagris gallopavo), Hybrid strain (French origin), were selected and kept for

42 days in the experimental pens. The poults were raised in concrete floor pens covered with 8 cm of clean pine wood shavings, and each pen was equipped with one tube feeder and one automatic waterer. Throughout the study, the poults were grown following standard temperature regimens, which gradually decreased from 38 ºC to 23 ºC and under 16L: 8D light schedule. Birds were allowed to consume feed and water ad libitum.

**Newcastle Vaccine:** One dose of vaccine containing 106.5 EID50 (50 percent Embryo Infectious Dose) of lentogenic freeze-derived live VG/GA strain of Newcastle disease vaccine (Merial Animal Health Limited, Lyon, France) was used in this study. All the turkey poults were vaccinated at age of 10 and 20 days by eye dropper according to the recommendations of the manufacturer. Blood samples were collected at 1, 9, 20, 28, 35 and 42 days of age. The blood samples pre first vaccination (days 1 and 9) were used for detection of maternal antibody level. Samples of pre second vaccination (day 20) were used to assess the IgY titers of the first vaccination, while the blood samples of days 28, 35 and 42 were utilized for evaluation of second vaccination humoral immune responses. Blood samples were immediately centrifuged to separate serum and stored at −20 °C until further analyses.

**Assessment of humoral immunity:** Specific antibody titer (IgY) against VG/GA Newcastle vaccine was measured using indirect enzyme-linked immunosorbent assay (ELISA) proposed by (Al-Karagoly et al., 2017) Al-Karagoly et al., (2017). Checkerboard titration was carried out to select the optimal concentration of coating antigen and antibody dilution. According to data obtained from checkerboard titration, a 96-well U-shaped polystyrene plate (Nunc, Roskilde, Denmark) was coated with 50µl of VG/GA antigen (7 µg/ml) in PBS (pH = 7.2) per well and incubated overnight at 4 °C. Plates were washed with PBS-T three times and nonspecific binding sites were blocked with 100 µl of PBS-Blotto, incubated for 1 h at room temperature. Plates were washed three times, and all tested sera were diluted in duplicate at 1:100 with PBS-T. Diluted (50 µl) sera were added into each well, at room temperature for 45 min, and then washed three times. Finally, 50 µl of diluted (1:10000) HRP-labeled goat anti-turkey IgY was added to each well and left for 30 min at 37 oC. After washing, TMB substrate was added and the mixture was incubated in dark place for 15 min at room temperature. The reaction was then stopped by the addition of 100 µl of 0.5 N sulfuric acid. The optical density (OD) was measured with an ELISA microplate reader (Stat FAX 2000, Awareness Technology, Inc., USA) at wavelengths of 450 nm.

**The Limit of detection (LOD):** The limit of detect (LOD) of IgY was expressed as the analytic concentration corresponding to the sample blank value plus three standard deviation as shown in the following equations:

$LOD=X_{b1}+3S_{b1}$

Where $X_{b1}$ is the mean concentration of the blank and $S_{b1}$ is the standard deviation of the blank.

**MHC genotyping:** DNA was extracted from whole blood samples using a Genomic DNA Extraction Kit (Bioneer, Korea). Second exon of MHC class II B locus (389bp) was amplified and used for High Resolution Melting analysis (HRM). Turkey MHC sequences (Genbank DQ993255 and EU522671) were used to design primers. Amplification was performed in a final

volume of 25 µL containing 20 ng template DNA, 1.5 mM MgCl2, 250 µM of each dNTP, PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 1 µl EvaGreen dye (20X), 1 U/µL of Taq DNA polymerase (CinaClon, Iran) and 20 pmol of specific primers (F: 5'-CTGCCCGCAGCGTTST-3'; R: 5'-CGAGACCCGCACCTTGG-3'). The thermal cycling profile was 1 cycle at 95 °C for 2 min, 30 cycles at 95 °C for 30 sec, 60.5 °C for 30 sec and 72 °C for 40 sec, with a terminal step of 5 min at 72 °C.

HRM curve analysis was performed in a Rotor-Gene™ 6000 thermal cycler (Corbett Life Science Pty Ltd). In order to determine the optimal melting condition for differentiation of MHC alleles, the PCR products were setup on 0.22 °C/s ramping between 75 °C and 95 °C. All specimens were tested and their melting profiles analyzed using Rotor-Gene 1.7 software and the HRM algorithm provided. Normalization regions of 80.47 - 80.97 and 89.71 - 90.21 were used for analysis. The HRM saturating fluorescent dye (Evagreen) was used in the PCR reaction to amplify the MHC class IIB exon2 (389bp). The Evagreen fluoresces strongly only when bound to dsDNA. The changes of the fluorescence during the test can be used both to measure the increase in DNA concentration during PCR amplification and, subsequently, to measure temperature- induced DNA dissociation during HRM. After PCR in the presence of the dsDNA-binding fluorescent dye (Evagreen), amplicons are briefly denatured and then rapidly reannealed. If the DNA sample is heterozygous, perfectly matched hybrids (homoduplexes) and mismatched hybrids (heteroduplexes) are formed. When the temperature is slowly increased again, the DNA begins to melt. The software HRMA system offers different methods of observing the data which assist with distinguishing genotypes even when the melting profile of each sample is similar. One of these methods explained in our study was the normalized HRM curve that was represented as the amount of fluorescence at each given temperature. The second analysis method involves plotting the fluorescence difference between normalized melting curves, called fluorescence difference curve. In this method, the genotype A is chosen as a reference and the difference between each curve and the reference is plotted against temperature. The reference curve becomes a horizontal line at zero and the other genotypes cluster along different paths for easy visual discrimination of the genotype classes.

**DNA sequencing:** The HRM method was used to detect genotypes and direct sequencing was applied to detect allele sequences and subtypes. Purification of PCR products and sequencing was performed by Macrogen Inc. (Seoul, Korea) on an ABI 3730 XL automatic DNA sequencer. Sequence analysis was achieved by NCBI, BLAST, EMBOSS Transeq, Chromas software version 2.5.1 (www.technelysium.com.au) and bioinformatics software BioEdit 7.0.5.3 (Hall 1999).

**Repeatability of the experiment:** The experiment was repeated several times to validate the results and a good repeatability as well as reproducibility was found.

**Statistical analysis:** The amount of gene diversity in the population was evaluated using by number of alleles (Na) and unbiased expected heterozygosity (Hexp), according to the formula proposed by (Nei, 1973). Deviation from Hardy-Weinberg equilibrium (HWE) was also estimated using likelihood ratio test in this population. Association of MHC IIB alleles with immune responses

**Table 1.** The MHC IIB allele frequency in commercial turkey poults (n=92).

| MHC IIB1 | Frequency (%) | MHC IIB2 | Frequency (%) | MHC IIB3 | Frequency (%) |
|---|---|---|---|---|---|
| 1*2 | 39.7 | 2*2 | 48.9 | 3*2 | 33.7 |
| 1*3 | 14.7 | 2*3 | 44.6 | 3*3 | 53.3 |
| 1*4 | 28.8 | 2*new | 6.5 | 3*4 | 13.0 |
| 1*5 | 16.9 | | | | |

**Table 2.** The Significant association of MHC II B alleles with humoral immune responses in commercial turkey poults (n=92).

| Immune traits | Vaccination | Study period (day) | Alleles | Allele effect | Std. Error | t | P value |
|---|---|---|---|---|---|---|---|
| NDV IgY | 1st | 20 | B1-1*4 | -99.166 | 46.559 | -2.130 | 0.036 |
| NDV IgY | 2nd | 35 | B1-1*3 | -101.209 | 45.863 | -2.207 | 0.030 |
| | | | B1-1*4 | -112.787 | 56.958 | -1.980 | 0.051 |
| NDV IgY | 2nd | 42 | B1-1*3 | -63.761 | 32.116 | -1.985 | 0.050 |
| | | | B1-1*5 | -62.884 | 31.025 | -2.027 | 0.046 |

against VG/GA vaccine was analyzed using the following model:

$Y_i=\mu+ \Sigma\ b_j f_{ij}+\varepsilon i$

Where $Y_i$ is a dependent variable for specific IgY response in ith turkey; μ is a general mean; $f_{ij}$ is the copy number of the MHC IIB jth allele in the ith turkey; $b_j$ is half the substitution effect for the MHC IIB jth allele; and εi is the residual effect for the ith turkey. Effects were considered to be significant when probability (P) values of ≤ 0•05 were obtained. The association study was evaluated using multivariate regression analysis and GLM procedures of SPSS software version 21.

## Results

**Humoral immune responses to Newcastle vaccine:** The maternal antibody level was checked at days 1 and 9 before first vaccination. At day 1 all the turkey poults showed a high IgY titer (ng/mL) (582.03±78.39) which dropped sharply after 9 days (303.43± 36.79). Based on the ELISA results, the IgY (ng/mL) mean titration value of vaccinated population was increased significantly after 10 days of the first vaccination (725.95±91.88). The level of IgY(ng/mL) continued to increase after the second vaccination until it reached the peak at 35 days of age (14 days after second vaccination) (811.33±119.46) and subsequently decreased at day 42 (21 days after second vaccination) (Fig. 1).

Our in-house ELISA assay had a good sensitivity to IgY concentration between 813 ng/mL and to 0.56 ng/mL. Our assay showed its detection limit of IgY in turkey at a concentration of 0.56 ng/ml. We found a detection limit of 0.56 ng/ml. It exceeds the sensitivity obtained by (Erhard et al., 1992). They demonstrated a detection limit of 20 ng TgY per ml in an assay based on monoclonal antibodies, also, higher sensitive than that obtained by (Fischer and Hlinak, 1996), which indicated a limit of detection of 0.78 ng/mL.

**MHC IIB genotyping:** HRM and Melt curve analysis demonstrated 8 heterozygote profiles (A, B, C, D, E, F, G and H) in 92 turkey poults that were also confirmed by sequence analysis. The heterozygous profiles were shown as the HRM normalized fluorescent curve (Fig. 2A) as well as the HRM fluorescence difference curve (Fig. 2B). Fluorescent signal from a heterozygous DNA

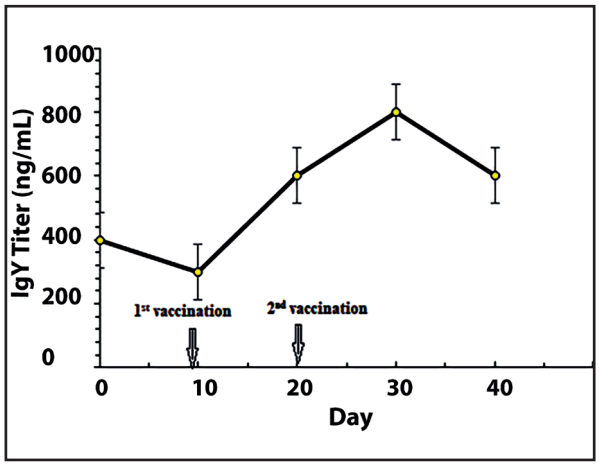


**Figure 1.** Least square means of NDV IgY antibody titers (ng/mL) in commercial turkey poults. (n=92).

sample shows a decrease in two characteristic temperatures, due to the different rates of strand separation in heteroduplex and homoduplex dsDNA. Thus, the shapes of melting curves obtained with homozygous and heterozygous samples, respectively, are significantly different. Profile D showed the highest (25%), and profiles C and G the lowest (6.52 %) frequency, respectively. Sequencing analysis also represented the presence of three MHC class II B loci (B1, B2 and B3) and 10 alleles (B1: 1*2, 1*3, 1*4, 1*5; B2: 2*2, 2*3, 2new; B3:3*2, 3*3, 3*4) including one new allele in this population. Allele B2*2 had the highest (48.9%), and alleles of B2* the lowest (6.5 %) frequency, respectively. Distributions of MHC class II B alleles are shown in Table 1. The observed heterozygosity (Hobs=100%) of MHC IIB loci was significantly greater than the expected (Hexp=56%) in this population ($P$=<0.01).

**Correlation between MHC alleles and humoral immune responses:** In total, significant correlation was observed between MHC IIB alleles with humoral immune responses to VG/GA Newcastle vaccine in commercial turkey poults ($P$<0.05). Significant association with IgY immune responses was made by MHC IIB1 locus. No significant associations were observed between MHC IIB2 and MHC IIB3 loci and antibody titer against NDV vaccine in commercial turkey poults ($P$> 0.05).

Significant negative correlation was detected between B1-1*4 allele and specific NDV IgY titer after 10 days of first vaccination (day 20) ($P\leq$ 0.036). Alleles B1-1*3 and B1-1*4 were also negatively correlated with specific NDV IgY titer after 14 days of second vaccination (day 35) ($P\leq$ 0.030 and $P\leq$ 0.051, respectively). Furthermore, alleles B1-1*3 and B1-1*5 were associated with lower antibody titers against NDV vaccine after 21 days of second vaccination (day 42) ($P\leq$ 0.05 and $P\leq$ 0.046, respectively) (Table 2).

## Discussion

Chicken major histocompatibility complex could be considered as a genetic marker associated with susceptibility or resistance to infectious diseases and immune responses against vaccines (Schou et al., 2010; Suzuki et al., 2010). Identifying the association between MHC haplotypes and improved immune responses against endemic diseases would be useful in resource conservation and genetic breeding strategies in animals(Chang et al., 2012).

In this study, we investigated the MHC IIB exon 2 polymorphism in commercial turkey poults (meleagris gallopavo) using HRM technique and DNA sequencing method. In total, 8 HRM patterns and 10 alleles were identified in this population. Allele B2-2*new was unique to turkey poults and has not been previously reported. Such unique alleles should be considered for conservation of genetic variation in populations. Results of present study indicated

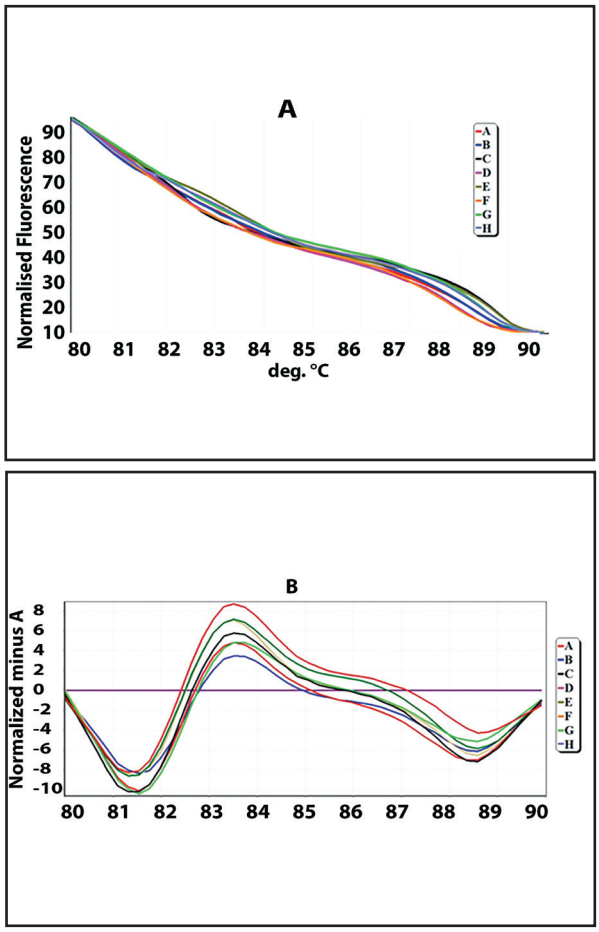


**Figure 2(A-B).** The HRM normalized fluorescence curve (Figure 2A) and fluorescence difference curve (Figure 2B) of eight MHC II B genotype profiles in commercial turkey poults. The nomenclatures of the eight HRM genotype profiles are (A, B, C, D, E, F, G, and H). A duplicate 92 samples were carried out by HRM analysis.

that HRM technique could be considered as an appropriate method for MHC genotyping in turkey, particularly when combined with DNA sequencing. HRM technique is able to detect homozygote or heterozygote genotypes in a simple, fast and inexpensive method (Wittwer et al., 2003). However, more powerful and accurate approaches such as sequencing might be necessary to confirm the exact number of alleles.

The association study revealed significant correlations between MHC IIB alleles with humoral immune responses to VG/GA Newcastle vaccine in commercial turkey poults. Three alleles (B1-1*3, B1-1*4 and B1-1*5) which belonged to the MHC IIB1 locus were significantly associated with lower antibody responses against NDV VG/GA vaccine. Several studies have presented the association between MHC genotypes and immune responses against Newcastle disease or vaccines in chicken (Esmailnejad et al., 2017; Ewald et al., 2007; Lwelamira et al., 2008; Norup et al., 2011); Esma. In particular, MHC class II alleles have been shown to be tightly linked to the antibody immune responses against Newcastle vaccine (Zhou and Lamont, 2003). Results obtained in this study were also in close agreement with the previous studies reported the association between MHC alleles and antibody titer against NDV vaccine in chicken.

The MHC is associated with resistance or susceptibility of chickens to disease organisms, including NDV (Dunnington et al., 1993). In evolutionary processes, MHC molecules could acquire a preference to exploit pathogen-specific signatures (Calis et al., 2010). The domestic chicken has been influenced to a large extent by some important pathogen-mediated selections (Chen et al., 2012), such as Newcastle disease virus and infectious bursal disease virus. The pathogen positive selection drives dominant trans-breeds B-L alleles that originate from ancestral genes. In addition, pathogen diversity has a role in shaping patterns of genetic variation at relevant MHC genes (Spurgin and Richardson, 2010). However, there is no information regarding the MHC genotypes polymorphism and their association with Newcastle disease resistance or susceptibility in commercial turkey poults. Understanding the genetic pattern of MHC in turkey and its association with important immune responses seems to be worthwhile regarding the genetic improvement of this

population.

The observations of this study indicated a high level of MHC IIB polymorphism and heterozygosity in commercial turkey poults (meleagris gallopavo). Significant correlations between MHC IIB1 locus and antibody level against VG/GA Newcastle vaccine were also detected. Three alleles reported in this study were associated with reduced immune responses against NDV vaccine in turkey population. Due to such negative associations, molecular breeding programs based on specific genetic markers should be implemented with great caution.

## Acknowledgments

This work was supported by the Tehran university research council and Iran national Science foundation, grant No. 93047261.

## Conflicts of interest

The author declared no conflict of interest.

## References

Alexander, D. J., Aldous, E. W., Fuller, C. M. (2012) A selective review of 40 years of Newcastle disease research. Avian Pathol. 41: 329-335.

Al-Karagoly, H., Nikbakht, G., Hassanzadeh, M., Tolouei, T. (2017) Turkey humoral and cell-mediated immune responses to a Newcastle viscerotropic vaccine and its association with Major Histocompatibility Complex. Bulg J Vet Med (BJVM). DOI: 10.15547/bjvm.2031

Briles, W. E., Stone, H. A., Cole, R. K. (1977) Marek's disease: effects of B histocompatibility alloalleles in resistant and susceptible chicken lines. Science. 195: 193-195.

Butter, C., Staines, K., Van Hateren, A., Davison, T. F., Kaufman, J. (2013) The peptide motif of the single dominantly expressed class I molecule of the chicken MHC can explain the response to a molecular defined vaccine of infectious bursal disease virus (IBDV). Immunogenetics. 65: 609-618.

Calis, J.J., Sanchez-Perez, G.F., Keşmir, C. (2010) MHC class I molecules exploit the low G+ C content of pathogen genomes for enhanced presentation. Eur J Immunol. 40: 2699-2709.

Chang, C. S., Chen, C. F., Berthouly-Salazar, C., Chazara, O., Lee, Y. P., Chang, C. M., Chang, K. H., Bed'Hom, B., Tixier-Boichard, M. (2012) A global analysis of molecular markers and phenotypic traits in local chicken breeds in Taiwan. Anim Genet. 43: 172-182.

Chaves, L.D., Faile, G.M., Kreuth, S.B., Hendrickson, J.A., Reed, K.M. (2010) Haplotype variation, recombination, and gene conversion within the MHC-B of the turkey. Immunogenetics. 62: 465–477.

Chen, F., Pan, L., Chao, W., Dai, Y., Yu, W. (2012) Character of chicken polymorphic major histocompatibility complex class II alleles of 3 Chinese local breeds. Poult Sci. 91: 1097-1104.

Dunnington, E., Stallard, L., Siegel, P., Hillel, J. (1993) Evaluating linkage between DNA fingerprint bands and quantitative traits in chickens: interactions. Poult Sci. 72: 1000-1004.

Emam, M., Mehrabani-Yeganeh, H., Barjesteh, N., Nikbakht, G., Thompson-Crispi, K., Charkhkar, S., Mallard, B. (2014) The influence of genetic background versus commercial breeding programs on chicken immunocompetence. Poult Sci. 93:77-84.

Esmailnejad, A., Nikbakht, Gh., Badavam, M. (2017) LEI0258 microsatellite variability and its association with humoral and cell mediated immune responses in broiler chickens. Mol Immunol. 90: 22-26.

Ewald, S. J., Ye, X., Avendano, S., McLeod, S., Lamont, S. J., Dekkers, J. C. (2007) Associations of BF2 alleles with antibody titres and production traits in commercial pure line

broiler chickens. Anim Genet. 38: 174-176.

Erhard, M., Von Quistorp, I., Schranner, I., Jüngling, A., Kaspers, B., Schmidt, P., Kühlmann, R. (1992) Development of specific enzyme-linked immunosorbent antibody assay systems for the detection of chicken immunoglobulins G, M, and A using monoclonal antibodies. Poult Sci. 71: 302-310.

Fischer, M., Hlinak, A. (1996) An ELISA for the quantification of chicken immunoglobulin (IgY) in various liquid media. Altex. 13: 179-183.

Hunt, H.D., Dunn, J. R. (2013) The Influence of host genetics on Marek's disease virus evolution. Avian Dis. 57: 474-482.

Hunt, H. D., Jadhao, S., Swayne, D. E. (2010) Major histocompatibility complex and background genes in chickens influence susceptibility to high pathogenicity avian influenza virus. Avian Dis. 54: 572-575.

Juul-Madsen, H. R., Dalgaard, T. S., Rontved, C. M., Jensen, K. H., Bumstead, N. (2006) Immune response to a killed infectious bursal disease virus vaccine in inbred chicken lines with different major histocompatibility complex haplotypes. Poult Sci. 85: 986-998.

Lwelamira, J., Kifaro, G., Gwakisa, P., Msoffe, P. (2008) Association of LEI0258 microsatellite alleles with antibody response against Newcastle disease virus vaccine and body weight in two Tanzania chicken ecotypes. Afr J Biotechnol. 7: 714-720.

Mishra, S., Kataria, J. M., Verma, K. C., Sah, R. L. (2000) Response of chickens to infection with Newcastle disease virus isolated from a guinea fowl. Trop Anim Health Prod. 32: 277-284.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci. 70: 3321-3323.

Nikbakht G, Esmailnejad A. (2015) Chicken major histocompatibility complex polymorphism and its association with production traits. Immunogenetics. 67(4):247-252.

Norup, L. R., Dalgaard, T. S., Pedersen, A. R., Juul-Madsen, H. R., (2011) Assessment of Newcastle disease-specific T cell proliferation in different inbred MHC chicken lines. Scand J Immunol. 74: 23-30.

Senne, D. A., King, D. J., Kapczynski, D. R. (2004) Control of Newcastle disease by vaccination. Dev Biol. 119: 165-170.

Schou, T. W., Labouriau, R., Permin, A., Christensen, J. P., Sørensen, P., Cu, H. P., Nguyen, V. K., Juul-Madsen, H. R. (2010) MHC haplotype and susceptibility to experimental infections (Salmonella Enteritidis, Pasteurella multocida or Ascaridia galli) in a commercial and an indigenous chicken breed. Vet Immunol Immunopathol. 135: 52-63.

Spurgin, L.G., Richardson, D.S. (2010) How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. Proceedings of the Royal Society of London B: Biological Sciences: rspb20092084

Suzuki, K., Matsumoto, T., Kobayashi, E., Uenishi, H., Churkina, I., Plastow, G., Yamashita, H., Hamasima, N., Mitsuhashi, T. (2010) Genotypes of chicken major histocompatibility complex B locus associated with regression of Rous sarcoma virus J-strain tumors. Poult Sci. 89: 651-657.

Taylor Jr, R.L. (2004) Major histocompatibility (B) complex control of responses against Rous sarcomas. Poult Sci. 83: 638-649.

Wittwer, C.T., Reed, G.H., Gundry, C. N., Vandersteen, J.G., Pryor, R.J. (2003) High-resolution genotyping by amplicon melting analysis using LCGreen. Clin Chem. 49: 853-860.

Zhou, H., Lamont, S.J. (2003) Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. Immunogenetics. 55: 133-140.

**مجله طب دامی ایران**، ۱۳۹۷، دوره ۱۲، شماره ۴، ۳۵۶-۳۴۷

# تنوع ژنتیکی MHC کلاس دو بوقلمون تجاری و ارتباط آن با پاسخ ایمنی هومورال

**حسن الکاراوقلی[۱] غلامرضا نیکبخت بروجنی[۱] عاطفه اسماعیل نژاد[۲] محمد حسن زاده[۳] توحید طلوعی[۳]**

**۱) گروه میکروبیولوژی و ایمنولوژی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران**
**۲) گروه پاتوبیولوژی، دانشکده دامپزشکی دانشگاه شیراز، شیراز، ایران**
**۳) گروه بیماری‌های پرندگان، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران**

(دریافت مقاله: ۱۴ اسفند ماه ۱۳۹۶، پذیرش نهایی: ۳۰ اردیبهشت ماه ۱۳۹۷)

## چکیده

**زمینه مطالعه:** مجتمع عمده پذیرش بافتی (MHC) یکی از نواحی ژنتیکی است که ارتباط آن با پاسخ ایمنی به واکسن بخوبی مشخص شده است. بررسی ارتباط هاپلوتیپ های MHC با پاسخ ایمنی بهتر برای طراحی واکسن و اصلاح نژاد کاربرد دارد.

**هدف:** ارزیابی تنوع ژنتیکی MHC کلاس دو بوقلمون تجاری و بررسی ارتباط آن با پاسخ ایمنی هومورال بر علیه واکسن نیوکاسل.

**روش کار:** تعداد ۹۲ جوجه بوقلمون گوشتی با واکسن VG/GA در سنین ۱۰ و ۲۰ روز واکسنه شدند. از روش الیزا برای تعیین پادتن اختصاصی IgY و از روش تحلیل دقیق دمای شکافت (HRM) و تعیین توالی برای بررسی تنوع ژن MHC کلاس دو استفاده شد. اثر آلل‌ها بر پاسخ ایمنی هومورال با آنالیز رگسیون چند متغیره و GLM صورت گرفت.

**نتایج:** ۸ پروفایل HRM و ۱۰ آلل در جمعیت شناسایی شدند. آلل‌های B۱-۱×۵، B۱-۱×۴، B۱-۱×۳ به طور معنادار با کاهش پاسخ هوموران بر ضد واکسن مرتبط بودند.

**نتیجه گیری نهایی:** سه آللی که در این مطالعه گزارش شدند با کاهش پاسخ ایمنی همراه بودند. به دلیل این ارتباط منفی برنامه‌های اصلاح نژاد بر مبنای شاخص ژنتیکی باید با دقت بیشتر انجام شوند.

**واژه‌های کلیدی:**
پادتن، ایمنی، MHC، بوقلمون، واکسن

نویسنده مسؤول: تلفن: ۶۱۱۱۷۰۵۷ (۲۱)۹۸+ نمابر: ۶۶۴۲۷۵۱۷ (۲۱)۹۸+ Email: nikbakht@ut.ac.ir