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Association between DRB3 gene Polymorphism and reproductive, immunity performance and heat tolerance in Holstein cow

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Abstract
This investigated was conducted at Al-Salam station for Dairy cattle /private sector, from 1-11-2016 to 1-11-2017, to determine the association between DRB3 gene's genotypes with some reproductive traits, immunity and heat tolerance coefficient in Holstein cows. The results DRB3 gene analysis showed a highly significant Different (P<0.01) between genotypes of DRB3 gene's genotypes A,B & C, the percentage were 46.00, 42.00 and 12.00% respectively. The results of the current study showed that services per conception and days open was significantly affected (P<0.05) by different genotypes of the DRB3 gene for the cows with A genotype. There was also a significant difference (P<0.05) between the genotypes of DRB3 gene for IgG concentration in calves blood. The concentration of IgG in this study 42.9± 2.39 & 42.75 ± 1.98 g/L. blood which belong to cows with A & B genotypes respectively and it was higher than claves which belong to cows with C genotype (38.08 ± 1.62 g /L. blood). For the heat tolerance coefficient it was found that there was a highly significant (P<0.01) difference between the genotypes of the DRB3 gene in this trait and for the cows with C genotype 105.65 ± 0.56 in the second month of the lactation season, and for the cows with A & B genotype (102.76 ± 0.63 and 103.05 ± 0.43 respectively), while there was no effect of DRB3 gene genotypes of the heat tolerance coefficient during the first and third months of the lactation season.
It was possible to Conclude from this study the possibility of DRB3 gen's genotypes in the development of genetic improvement strategies and in breeding programs in dairy cows.

Keywords: Holstein cows, Reproductive, Immunity performance- Heat tolerance- DRB3 gene

1. Introduction
The livestock production sector is an important in the economies of countries including Iraq because of its role in food security, which contributing about 40% of the value of agricultural products [1], There has been a deterioration in the animals production sector in general and cattle in particular and the decline in the number of farm animals compared with the population increase in recent years [2], and the infection of animals with infectious diseases lead to decrease in reproductive performance and this leads to increase in veterinary costs and therefore high production costs The costs associated to these types of problems, are mainly represented by the decrease in milk production, veterinary costs, premature discard of animals, milk rejection due to antibiotic contamination, among others [3], Therefore, the researchers resort to perform the traditional genetic improvement of the agricultural animals in general and cattle especially, which relied on statistical methods and focused on the selection of individuals with a better phenotypic structure, which achieved significant gains in the field of genetic improvement, but scientific acceleration and the availability of large information on the work of the genome has enabled to set a selection programs more accurate and less time-consuming and cost, economic characteristics were controlled by a number of genetic loci known as quantitative sites (QTL- Quantitative trail loci) it is possible to predict the phenotype variation of the traits by identified these loci and associated genetic markers with it to be improved early and to build the selection programs on them, These markers are functional mutations in the genes affecting traits and resistance to infectious disease [4], DRB3 gene is one of the Major Histocompatibility complex type –A genes (MHC-Class II) which belonged to Immunoglobulin super family and these genes were a glycoproteins and it's one of the major histocompatibility complex genes had more polymorphism [5], it was located on the short arm of chromosome 23 in cattle [6].

Aim of this study was to determine the polymorphism of DRB3 gene and elicitation Distribution rate of that polymorphism and allelic frequency and it association with some reproductive traits and immunity and heat tolerance coefficient.

2. Materials and methods

This study was conducted in Al-Salam station for Dairy cattle /private sector (Al-Latifia district 25 km southern Baghdad), from 1-11-2016 to 1-11-2017, on 50 Holstein cows and their 50 offspring, for DNA extraction and DRB3 gene analysis in were carried out in Al-Takadom scientific lab to determine the association between polymorphisms of DRB3 gene and it's rate and allelic frequency with some reproductive traits(services per conception and days open), immunoglobulin's concentration (IgG) and heat Tolerance coefficient for the lactation season 2016- 2017.

Blood collected by a medical syringe from the jugular vein in a 15 ml sterile polypropylene tubes containing 0.5 ml of EDTA (0.5 M) as an anticoagulant by the phenol chloroform extraction by the veterinarian at the station, The blood samples were then transferred by a cool box then stored in freezer at -20 ◦C temperature till transferred to the lab to extracting DNA, for the calves blood also collected by medical syringe from the jugular vein in a 10 ml tubes, the DNA samples were checked for their quality, purity and concentration, the quality of the genomic DNA was checked by using agarose gel electrophoresis, DNA samples of good quality, purity and concentration were used for further analysis. The polymerase chain reaction (PCR) technique for DRB3 typing is based upon the extensive polymorphism that is present in exon 2 of the DRB3 gene under consideration depending on the size of the pieces and type of primers used, The 284 bp fragment consisting [7] of the 267 bp exon 2 region of the DRB3 gene and the flanking intron of 17 bp present in the genomic DNA of cattle was amplified by employing the corresponding primer pairs (forward and reverse) as described by [8], The details of the primer sequences are as follows:

F: ATCCTCTCTCTGCAGCACATTTCC
R: TCGCCGCTGCACAGTGAAACTCTC

After the polymerase reaction was completed, the polymorphism of DRB3 gene were identified in blood samples from the cows after proceeding the cutting to the required piece of gene (284 bp) by restriction enzyme *HeaIII* from Haemophilus aegyptius bacteria, The digestion with *HaeIII* revealed three restriction sites, which resulted in three pieces (167, 65, 52 bp) for *HeaIIIa* and two pieces (219, 65 bp) for *HeaIIIb* and (167,117) for *HeaIIIc*. This restriction enzyme was obtained by the American Promega Company, the concentration of enzyme was 2500 U, 10u-l µ.

For the heat tolerance coefficient (HTC) it was calculated according to [9] equation:

HTC = 100 – [18(RT-39.1)]

As the:

18:fixed.

RT: average of rectal temperature at morning and afternoon.

39.1: normal of rectal temperature (centergate)

The data was analyzed by Statistical Analysis System [10] to study the polymorphism of DRB3 gene according the mathematical model, significant differences was compared by least square means method.

Yijk = µ + Gi + Oj + eijk

Yijkl : observed value K which belongs to phenotype i and month of birth j

µ: general mean

Gi: effect of DRB3 polymorphism (A,B,C)

Oj: effect of month of birth (April, may, June)

eijk: Random error which distributed normally with mean= 0 and variation σ2e

Chi-square- χ2 test were used to compare between the percentages of DRB3 gene polymorphisms.

3. Results and Discussions

The polymerase chain reaction (PCR) technique was used to extract DRB3 gene, PCR kit, primers of the gene and total DNA samples were used in a final volume of 5 µl and resolved on 2% agarose gel electrophoresis at 100 volt for 70 minutes in 1×TBE buffer and documented through photography using a gel documentation system to ensure from DNA extraction succeed and got 284 bp of the required piece of DRB3 gene, and used 1000 bp DNA ladder to estimate the size of the fragments as the figure no.1.

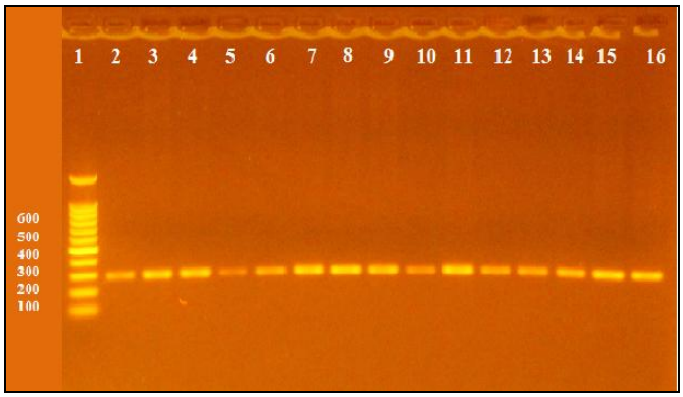

Fig 1: DRB3 gene extracted by Polymerase chain reaction method, column no.1 represented DNA Ladder 1000bp, column no.2-16 represented DRB3 gene piece amplified with Polymerase chain reaction method

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique and restriction enzyme *HeaIII* used to identify DRB3 gene polymorphism according to the method that mentioned in material and methods, the restriction fragments were resolved on 2% agarose gel electrophoresis at 100 volt for 70 minutes in 1×TBE buffer and documented through photography using a gel documentation system to ensure from DNA extraction succeed and got 284 bp of required piece, and the results of analysis showed three allelic: allelic A (219,167,52)bp, allelic B (167,52)bp, allelic C (219,52)bp, as the figure no.2.

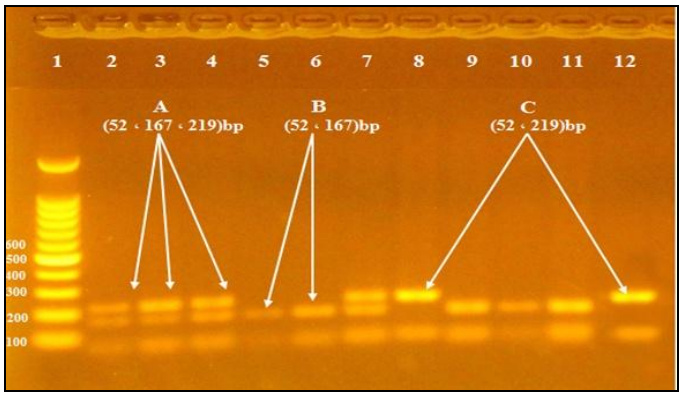

Fig 2: DRB3 gen three genotypes identified as the size of bands, column no.1 represented DNA Ladder 1000bp,column no.2,3,4,7 represented A allelic (219,167,52)bp, column no. 5,6,9,10,11 represented B allelic (167,52)bp, column no. 8,12 represented C allelic (219,52)bp with 17 bp not shown when compare it with gene sequence in NCBI (wild gene)

Table (1) showed the percentage of DRB3 gene polymorphism, there were a highly significant difference (P<0.01) between the gene polymorphism which reached to 46.00, 42,00 & 12,00% for A,B & C allelic respectively, there was a common for genotype A then B if compared with C genotype, these results are proof that DRB3 gene primer we used in this study was really exist in the genome of the Holstein cattle, the results of the previous studies indicated that there are highly significant differences (P<0.01) in DRB3 gene polymorphism [11, 12, 13]. The prevalence of allelic A and B and Scarcity of allelic C in this study maybe due to the adapted of first and second allelic to the environmental conditions in central Iraq that Holstein cattle lived from high temperatures for most months of the year and scarcity of rainfall and deficiency in nutrition or because animals depended roughages on the feed therefore perhaps natural selection plays a role against the C allelic.

Table 1: Number and percentage for DRB3 gene polymorphism

Polymorphism	Number	Percentage %
A	23	46.00
B	21	42.00
C	6	12.00
Total	50	100%
Chi-square- χ^2 value	----	10.175 **
(P<0.01) **		

Association of DRB3 gene with services per conception and days open

Table (2) showed the association between DRB3 polymorphism and services per conception and days open, there was a different significant (P<0.05) in both traits when the genotypes was different, cows with B genotype needed minimal services per conception (1.48 ± 0.14) and thus less days open (81.46 ± 5.32 day), while the rates of these two traits were 1.79 ± 0.13 & 90.50 ± 5.34 day, 1.75 ± 0.27 & 91.13 ± 10.46 day in A and C genotypes respectively, the reason may be due to the association between major histocompatibility complex allelic and the resistance and sensitivity against pathogens or maybe due to its positive effect to fight bacterial infections [14], these two traits were the most important indicators of reproductive efficiency in dairy cows because they reflect the number of births during lactation season and the physiological and health status of the cow and herd management and attention to the fertilization process and thus the number of births during productive life, which determines the economic return of cattle breeding projects and the possibility of applying the selection programs.

Table 2: Association between DRB3 gene polymorphism and services per conception and days open

Polymorphism	Cows number	Mean ± Standard error	
		Services per conception	Days open
A	23	1.79 ± 0.13 a	90.50 ± 5.34 a
B	21	1.48 ± 0.14 b	81.46 ± 5.32 b
C	6	1.75 ± 0.27 a	91.13 ± 10.46 a
Total	50	*	*
Significantly			

The means with different letters within the same column are significantly between them (P<0.05) *

Association of DRB3 gene with Immunoglobulin's concentration

Table (3) showed that there was a different significance (P<0.05) between the genotypes derived from the analysis of DRB3 gene for cows for IgG concentration in calves blood, as the concentration of IgG in this study reached 42.92 ±2.39 & 42.75 ± 1.98 g /L calves blood derived from cows with A, B genotype respectively, while the concentration of IgG Decreased in blood of claves Belongs to cow with C genotype.

Table 3: Association between DRB3 gene polymorphism and Immunoglobulin's concentration

Polymorphism	Cows number (samples)	Mean ± Standard error
		Immunoglobulin's Concentration (g /blood) calves
A	23 (46)	42.92 ± 2.39 a
B	21 (42)	42.75 ± 1.98 a
C	6 (12)	38.08 ± 1.62 b
Total	50 (100)	*
Significantly		
The means with different letters within the same column are significantly between them, (P<0.05) *		

Association of DRB3 gene with Heat tolerance coefficient

Observed from table (4) a highly different significant (P<0.01)between DRB3 gene genotypes and heat tolerance coefficient, It was found that there was a highly significant (P<0.01) difference between the genotypes of the DRB3 gene in the heat tolerance coefficient and for cows with C genotype in the second month of the lactation season, the heat tolerance coefficient was 105.65 ± 0.56 for the cows carrying the genotype A & B (102.76 ± 0.63 and 103.05 ± 0.43 respectively), while there was no effected of DRB3 gene genotypes of the heat tolerance coefficient of cows during the first and third months of the lactation season, this may be due to the fact that disease is a stressful factor for living organisms, and since the cluster functions of the first and second type of major histocompatibility complex was the speed, specialization and effectiveness, heat shock proteins facilitate molecular processes and serve as targets for the immune system in healthy individuals, they consider themselves the dominant antigens on a wide range of pathogens, play a role in the immune response and act as an early warning of the body's immune system [15].

Table 4: Association between DRB3 gene polymorphism and heat tolerance coefficient

Polymorphism	Cows number (samples)	Mean ± Standard error for heat tolerance coefficient		
		1st month of lactation season	2nd month of lactation season	3rd month of lactation season
A	23(46)	102.33 ± 0.82 a	102.76 ± 0.63 b	103.28 ± 0.39 a
B	21(42)	103.19 ± 1.06 a	103.05 ± 0.43 b	104.94 ± 0.61 a
C	6(12)	102.94 ± 0.58 a	105.65 ± 0.56 a	103.89 ± 0.49 a
Total	50(100)	N.S	*	N.S
Significantly				
The means with different letters within the same column are significantly between them, (P<0.05) *, NS= No significant				

4. Conclusion

We can conclude from this study that cows with B genotype had better services per conception and days open, while the cows with A&B genotype gave the best levels of immunoglobulin's in their calves blood when compared it with C genotype, for the heat tolerance coefficient we found that difference in DRB3 gene genotype was significant during the second month of the lactation season.

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