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Polymorphisms of *TLR4* Gene and Its Association With Genetic Resistance to *Salmonella Enteritidis* Infection in *Fayoumi* Breed and *Hy-line* Strain in Egypt

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ABSTRACT

The aim of this study is to investigate the polymorphism in exon1 and 2 of *TLR4* gene and its possible association with resistance to salmonella infection in the *Fayoumi* breed and *Hy-line* strain of chickens and its possible association with immune response of birds under study. Two experiments were done, in which the chicks were infected intraesophageal with two different doses of *S. enteritidis* LD_{50} (10^8 cfu) in experiment I and 1/2 LD_{50} (5×10^7 cfu) in experiment II. The birds (100 chicks from each breed) allocated into different groups according to the clinical signs in experiment I to susceptible and resistant and in experiment II, to high and low immune response groups based on ELISA test. *TLR4* gene was genotyped by PCR-RFLP of exon 1 (596 bp) and exon 2 (793 bp) using different restriction enzymes. Comparison between the different genotypes generated by *Taq1* of *Fayoumi* breed and *Hy-line* strain in experiment I and II give valuable result. Exon 1- *Taq1* AA genotype can be used as a marker for culling the birds of *Hy-line* strain as it appears in low immune response and also, BB genotype (0.7) in susceptible birds. Moreover, using exon 2- *EcoR-1* BB genotype (0.9) in susceptible birds as a marker for culling these birds. We concluded that the Polymorphisms of *TLR4* - exon 1 and 2 can be used as a marker-assisted selection for resistant and/or susceptibility to salmonella infection in *Hy-line* strain.

1. INTRODUCTION

The chicken provides a major protein source from meat and eggs for most human populations throughout the world (Wicker et al. 2005). *Fayoumi* is a layer breed originated in Egypt, characterized by high immunity, high adaptation to Epyptian local conditions and good egg quality, while its production traits are low (Fimland, 2007; Taha *et al*., 2012 and Radwan, 2015). *Hy-line* is a foreign strain of the White *Leghorn* breed characterized by heavier body weight, deepest body, longest shank, taller comb, wider wattle lengths and get earlier sexual maturity compared with other breeds (Emara et al. 2003). It's characterized by a higher percentage of egg production such as egg number, weight and mass than other layer chickens (Radwan et al. 2015).

In the livestock farming, diseases have a severe effect not only on the performance of livestock but also on the quality of animal products that affect human health. One of the most hazardous disease in poultry farm is *salmonella* infection, which affected human health. *Salmonella enterica* is one of the most causes of food poisoning in humans, through infected poultry products (Rabsch et al. 2001). Salmonellosis in young chicken is a major disease characterized by severe signs of diarrhea, dehydration and a high mortality rate, resulting in economic losses in the poultry industry (Bumstead and Burrow, 1988; Bryan and Doyle, 1995 and Wigley et al., 2005).

In general, the immune system consists of genetic, molecular and cellular components that interact with each other, forming a complex communication

network. As a genetic component, the immune system was controlled by many genes. The polymorphisms in genes involved in chicken immune function have been associated with resistance to *salmonella enteritidis* challenge or vaccination (Lamont et al., 2002; Liu and Lamont, 2003and Malek et al. 2004).

Toll-like receptor 4 (*TLR4*) gene is a phagocyte cell surface receptor that plays a role in recognition of lipopolysaccharide (LPS) of gram-negative bacteria including *salmonella enteritidis* (Akira and Takeda, 2004). It is the most important markers showing a resistance of chicken against *salmonella* (Ulupi et al. 2013).

The peak response of antibody-producing cells from *salmonella* infected chickens, assayed by solid-phase ELISA occurred at 3 weeks post inoculation (Hassan et al. 1991). However, no data are available about the breed variation in delayed immune response to *salmonella* infection.

The aim of this study is to screen for the polymorphisms in *TLR4*-exon 1 and 2 and its possible association to *salmonella enteritidis* infection in *Fayoumi* breed and *Hy-line* strain of chicken based on the phenotypic classification of the birds either their clinical signs following acute infection or on antibody titer using ELISA in delayed response to smaller dose of *salmonella enteritidis* infection.

2. MATERIALS AND METHODS

2.1. Birds

Two hundred, one day old male *Fayoumi* and *Hy-line* strain chicks (100 per each breed) were used in this study. The birds were fed a starter feed from 1-7 days old and grower feed from 8-45 days old. The chicks received a normal vaccination regime and optimum housing conditions. All birds were handled in accordance with the recommendations of the committee on the ethics of Animal Experiments of Faculty of Alexandria Univerisity, Egypt.

2.2. Experimental infection and sample collection

2.2.1. Experiment I

At the age of two weeks, the chicks were infected intraesophageal with a high dose of *salmonella enteritidis* (10^8 cfu/bird) which represents LD_{50} (Ishola et al. 2008). After 48 hrs post challenged**,** the birds were allocated into two subgroups based on clinical signs which include diarrhea, ruffled feathers, inappetence,nthirst and reluctance to move (susceptible) and other birds which appear apparently normal (resistant). These birds Blood samples were collected from wing vein of 20 birds form each group at one week post infection into vacationer tubes containing an anticoagulant ethylene diamine tetra acetic acid (EDTA). The samples were stored at -20^{o}C until further use in DNA extraction.

2.2.2. Experiment II

At two weeks of age, the chicks were infected intraesophageal with a low dose of *salmonella enteritidis* (1/2 LD_{50}) which equal to 5×10^7 cfu (Woodward et al. 2002) for measuring the antibody titer. All the birds were tagged with wing tag, then after three weeks of infection, the blood samples were collected for ELISA test for measuring the antibody titer. According to the ELISA results, the birds were allocated to a high immune response (high antibody titer) and low immune response (low antibody titer). Blood and tissue samples were collected in the same manner as in experiment I.

Table 1: Primer sequences used for amplification of *TLR4* gene.

Genes	Primer sequence (5'–3')	Amplion Size (bp)	Accession No.
***TLR4*-Exon 1**	F: GAAACGTTGTCAGAGGTTCCTATG R: ACTTTGGTCCACCCATACTAATTT	596	NM_001030693.1
***TLR4*-Exon 2**	F: TGTTTCATCCACATTTACCCTCTT R: TCTTCCATTCCAGATGTTTCACT	793	NM_001030693.1

2.2.2.1. ELISA (Solid-phase Enzyme-Linked Immunosorbent Assay)

Collected blood samples three weeks after infection with *salmonella enteritidis* (5×10^7 cfu) were centrifuged at 4000 rpm for 10 minutes to separate serum samples. ELISA test was done according to Combs et al. (1980).

2.2.3. DNA extraction and Amplification of the *TLR4* gene.

DNA was extracted from experimental samples using Blood-Animal-Plant DNA Preparation Kit (Thermo scientific, Lithuania), according to manufacturer's instructions. The PCR was done for amplification of *TLR4* gene exon 1 and 2 by using primers shown in table (1).

TLR4 gene was amplified in 25 µl reaction volume. The reaction containing 2.5 µl 10X buffer, 0.5 µl of dNTPs mix, 0.5 µl of each primer (10 Pmol), 0.5 µl Taq DNA polymerase 500 units/µl (Thermo scientific, Lithuania), 2 µl genomic DNA and 18.5 µl dH_2O. The PCR program was carried out by initial denaturation at 95°C for 5 min followed by 35 cycles of 94 °C for 1 min for DNA denaturation, annealing temperature 58 °C (Leveque et al. 2003) for 1 min and extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified PCR products of *TLR4* gene was separated on 2 % ethidium bromide stained agarose gel, and photographed by Gel documentation system (Gel Doc, Alpha-Chem, Umager, USA). The PCR products stored at -20 °C.

2.2.4. Restriction Fragment Length Polymorphism (RFLP)

The PCR product was digested using restriction enzymes. The RFLP using *MSP1* restriction enzymes (Thermoscientific, Lithuania) was carried out in 15 µl reaction volume consist of 5 µl PCR product, 1µl green buffer, 8 µl dH_2O and 1 µl of fast digest *MSP1* restriction enzyme. Then incubated at 37 °C for 5 min using a thermal cycler (Technee, TC-3000, and USA). While, the RFLP in case of *EcoR1, Taq1* and *Hha1* restriction enzymes (Promega, USA) were carried out in 20 µl reaction volume consist of 5 µl PCR product, 2µl 10x buffer, 12.3 µl dH_2O, 0.2 µl acetylated bovine serum albumin 10 mg and 0.5 µl from any restriction enzyme (*EcoR1*, *Taq1* or *Hha1*), Then centrifuged few second and incubated at 37 °C for 3 hrs using a water bath. The cut products were loaded on 3% ethidium bromide stained agarose gel with and photographed by Gel documentation system (Gel Doc, Alpha-Chem, Umager, USA). Genotype frequencies of *TLR4* gene in the *Fayoumi* breed and *Hy-line* strain were estimated by direct counting.

2.2.5. Statistical analysis

Fisher's exact test was used to study the significant differences between different genotypes in studying breeds and susceptibility, resistant, low immune and high immune response birds. ANOVA was used to study significant differences in ELISA at $P\leq 0.05$.

3. RESULTS

3.1. Phenotypic classification of birds

In experiment I, a phenotypic classification of the birds into resistant and susceptible groups was done in the acute infection based on clinical signs which include diarrhea, ruffled feathers, inappetence, thirst and reluctance to move (susceptible) and other birds which appear apparently normal (resistant). In experiment II, based on ELISA titer after three weeks of infection the birds of both breeds were classified into high and low immune response. In *Fayoumi* breed, high antibody titer is ranged from 2.01 to 2.68 and low titer is ranged from 1.65 to 1.99. In *Hy-line* strain, high antibody titer is ranged from 1.80 to 2.78 and low titer is ranged from 0.97 to 1.59.

ELISA titer indicates a significant difference in antibody titer in *Fayoumi* breed compared with *Hy-line* strain. Also, a significant difference was observed between high and low antibody titer within the same breed of chicken. Differences in ELISA results among the *Fayoumi* breed and *Hy-line* strain are shown in table (2).

3.2. PCR amplification of *TLR4* Gene

The genomic DNA from *Fayoumi* breed and *Hy-line* strain of chickens (20) from each group in experiment I (susceptible and resistant), experiment II (high and low immune response) were used to amplify the *TLR4*- exon 1 which yields a fragment of 596 bp and exon 2 (793 bp) as shown in figure (1).

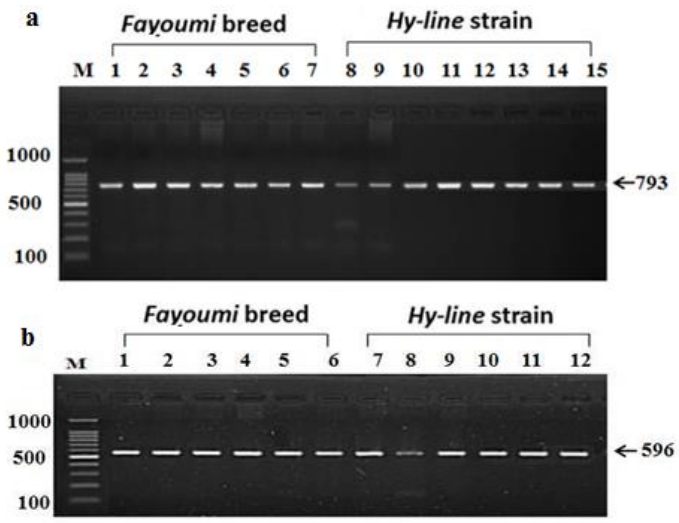

Figure 1: Ethidium bromide stained agarose gel of PCR products (a) representing amplification of *TLR4* gene- exon-1 with band size 596 bp. (b) representing amplification of exon 2 with band size 793 bp. Lane M: 100-bp DNA ladder.

3.3. Restriction Fragment Length Polymorphism (RFLP) of *TLR4* - exon 1 and exon 2 and genotype frequencies.

3.3.1. Experiment I

This experiment was used to associate with resistance to *salmonella* infection and polymorphisms in *TLR4* gene based on clinical signs.

3.3.1.1 Toll-like receptor 4 (*TLR4*) - exon 1

Restriction analysis of PCR-RFLP of *TLR4*- exon1 (596 bp) produced one uncut fragment (596 bp) by *MSP1*, *EcoR1*, and *Hha1* restriction enzymes in susceptible and resistant birds from *Fayoumi* breed and *Hy-line* strain producing one genotype (AA). While the effect of *Taq1* restriction enzyme on *TLR4*-exon1 (596 bp) in the *Fayoumi* breed and *Hy-line* strain revealed two genotypes in susceptible birds (Figure 2a) and resistant birds (Figure 2b). Genotype BB with two bands (460 bp and 136 bp) and genotype AB with three bands (596 bp, 460 bp and 136 bp) while AA genotype is absent (Figure 2).

In *Fayoumi* breed, the gene frequencies of susceptible birds were 0.4 BB and 0.6 AB while in resistant birds were 0.5 BB and 0.5 AB as shown in table (4). Regarding *Hy-line* strain, the gene frequency of susceptible birds was 0.7 BB and 0.3 AB. In resistant birds of *Hy-line* strain the gene frequencies was 0.5 BB and 0.5 AB. In *Hy-line* strain, the genotype BB (0.7) can be used as a marker-assisted selection for culling the susceptible birds (Table 3).

3.3.1.2 Toll-like receptor 4 (*TLR4*) - exon 2

Restriction analysis of amplified *TLR4*-exon 2 (793 bp) produced one uncut fragment 793 bp by *MSP1*, *Hha1*, and *Taq1* restriction enzymes in susceptible and resistant birds in the *Fayoumi* breed and *Hy-line* strain. So, one genotype (AA) was produced. The effect of *EcoR1* restriction enzyme on *TLR4*-exon 2 (793 bp) in the *Fayoumi* breed resulting one genotype BB with two bands (560 bp and 233 bp) in all birds (susceptible and resistant) as shown in figure (3).

Restriction analysis of PCR products of *TLR4*-exon 2 (793 bp) with *EcoR1* in the *Hy-line* strain revealed two genotypes in susceptible and resistant birds. The BB genotype with two bands (560 bp and 233 bp) and AB genotype with three bands (793 bp, 560 bp and 233 bp) while, AA genotype is absent (Figure 4).

The *EcoR1* restriction enzyme produced only genotype BB with two bands (560 bp and 233 bp) in the *Fayoumi* breed as shown in table (4). While, *Hy-line* strain with *EcoR1* revealed 0.1 gene frequency of AB in susceptible birds and 0.5 in resistant bird and 0.9 of BB genotype in susceptible birds and 0.5 in resistant birds. This means that BB genotype is more frequent in susceptible birds as it can be used as a marker for culling the susceptible birds and AB genotype is more in resistant birds (Table 4).

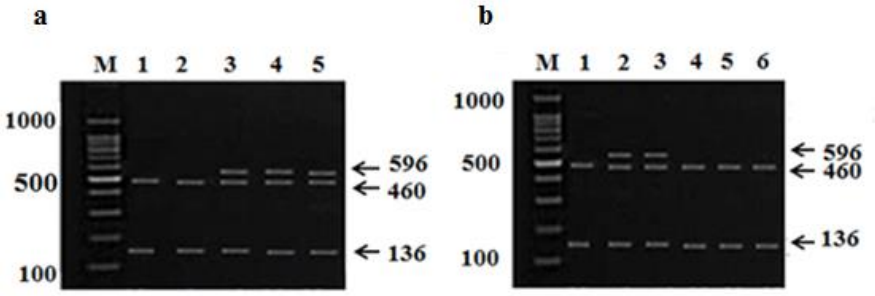

Figure 2: *Taq1* restriction fragment pattern of *TLR4*-exon1 (596 bp) in *Fayoumi* breed and *Hy-line* strain. Susceptible (a), Resistant (b). In a, lane1 and 2=BB genotypes and lane 3, 4 and 5=AB genotypes. In b, lane 1, 4 and 6=BB genotypes and lane 2 and 3 represent AB genotypes. Lane M: 100-bp DNA ladder.

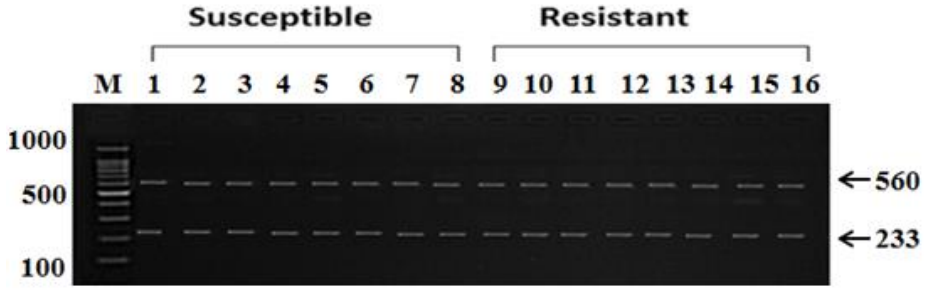

Figure 3: *EcoR1* restriction fragment pattern of *TLR4*-exon 2 (793bp) in *Fayoumi* breed. Lane (1-8) susceptible and lane (9-16) resistant. Showed only BB genotype (560 bp and 233bp). Lane M: 100-bp DNA ladder.

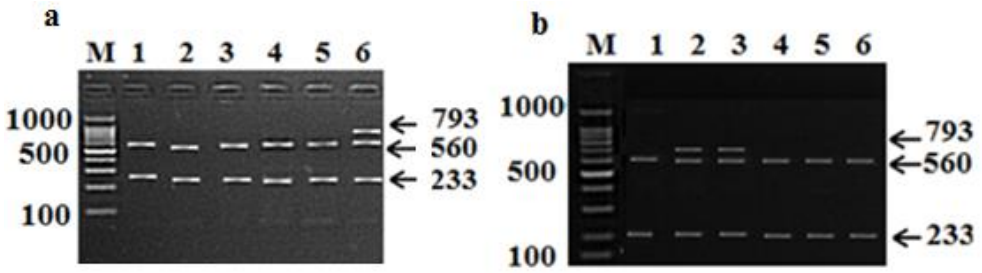

Figure 4: *EcoR1* restriction fragment pattern of *TLR4*-exon 2 (793 bp) in *Hy-line* strain. Susceptible (a), resistant (b). In a, Lane 1-5 = BB genotypes and lane 6 represent AB genotypes. In b, lane 1, 4, 5 and 6 = BB genotypes, lane 2 and 3 = AB genotypes. Lane M: 100-bp DNA ladder.

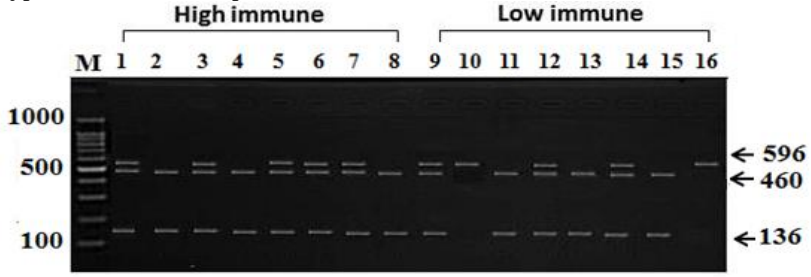

Figure 5: *Taq1* restriction fragment pattern of *TLR4*-exon 1 (596 bp) in *Fayoumi* breed and *Hy-line* strain. High immune response in lane 1-8, low immune response in lane 9-16. Lane 10 and 16 represent AA genotypes, lane 2,4,8, 11, 13 and 15 represent BB genotypes and lane 1, 3, 5, 6, 7, 9, 12 and 14 represent AB genotypes. Lane M: 100-bp DNA ladder.

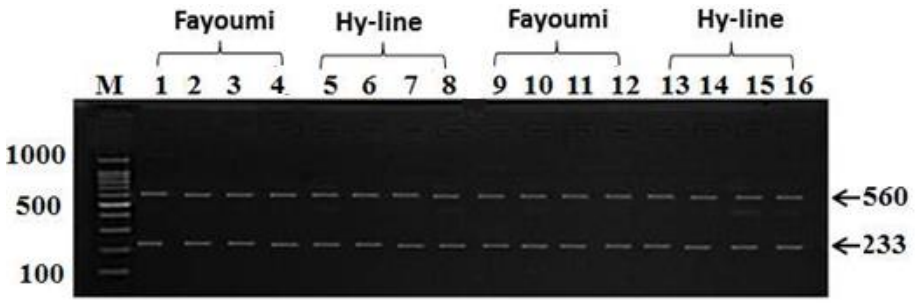

Figure 6: *EcoR 1* restriction fragment pattern of *TLR4*-exon 2 (793bp) in *Fayoumi* breed and *Hy-line* strain. Lane 1-4 *Fayoumi* high immune response, lane 9-12 *Fayoumi* low immune response. Lane 5-8 *Hy-line* high immune response and lane 13-16 *Hy-line* low immune response. Showed only BB genotype (560 and 233bp). Lane M: 100-bp DNA ladder.

3.3.2. Experiment II

This part used to evaluate the variation in immune response to a smaller dose of *salmonella* infection and its association to the polymorphisms of *TLR4*-exon1 and 2.

3.3.2.1. Toll-like receptor 4 (*TLR4*) - exon 1

The PCR-RFLP of *TLR4*-exon 1 (596 bp) produced one fragment (596 bp) by *MSP1*, *EcoR1* and *Hha1* restriction enzymes in high and low immune response chickens from *Fayoumi* breed and *Hy-line* strain giving one genotype (AA). The effect of *Taq1* restriction enzyme on *TLR4*-exon1 (596 bp) in the *Fayoumi* breed and *Hy-line* strain revealed three genotypes, The AA genotypes with uncut fragment (596 bp), the BB genotypes with two bands (460 bp and 136 bp) and AB genotypes with three bands (596 bp, 460 bp and 136 bp) as in figure (4).

In *Fayoumi* breed, the gene frequencies of high immune response birds were 0.4 with AA and 0.6 with AB. In low immune response birds, The AA, BB, and AB were 0.1, 0.5 and 0.4 respectively (Table 5). For *Hy-line* strain the gene frequency in high immune response birds was zero, 0.4 and 0.6 for AA, BB and AB respectively. However, in low immune response birds the gene frequencies were 0.3, 0.4, and 0.3 for AA, BB, and AB respectively (Table 5).

Comparison, between the different genotypes with *Taq1* of the *Fayoumi* breed and *Hy-line* strain in experiment I (susceptible and resistant) and experiment II (high and low immune response), the genotype of birds depending on immune response give valuable results. Where AA genotype (0.3) can be used as a marker for culling the birds in *Hy-line* strain as it appears only in low immune response. Moreover, the frequency of BB genotype is more in susceptible birds in *Hy-line* strain (0.7) as shown in table (6).

3.3.2.2. Toll-like receptor 4 (*TLR4*) - exon 2

Restriction analysis of amplified *TLR4*-exon 2 (793 bp) produced one uncut fragment (793 bp) by *MSP1*, *Hha1* and *Taq1* restriction enzymes in high and low immune response chickens from *Fayoumi* breed and *Hy-line* strain, giving one genotype (AA). The effect of *EcoRI* restriction enzyme on *TLR4*-exon 2 (793 bp) in the *Fayoumi* breed and *Hy-line* strain resulting in one genotype BB with two bands (560 bp and 233 bp) in the *Fayoumi* breed and *Hy-line* strain either high or low immune response birds (Figure 6). In *Hy-line* strain, genotype results depending on clinical signs are more valuable as BB genotypes (0.9) which appears in susceptible birds can be used as a marker for culling these birds (Table 7).

Table 2: Analysis of variance of ELISA test between and within breed based on antibody titer.

Chicken	Immune status	Number	ELISA- level Mean±SE
***Fayoumi* breed**	High response	20	2.38 ± 0.05^{a}
	Low response	20	1.86 ± 0.04^{c}
***Hy-line* strain**	High response	20	2.19 ± 0.09^{b}
	Low response	20	1.26 ± 0.06^{d}
	Total	80	2.00±0.07

Table 3: Genotype frequency of *TLR4* - exon 1 with *Taq1* restriction enzyme in experiment I.

Chickens type	Genotype frequency		
	AA	BB	AB
***Fayoumi* breed**			
Susceptible	Zero	8/20 = 0.4	12/20 = 0.6
Resistant	Zero	10/20 = 0.5	10/20 = 0.5
Fisher's Exact test	**0.7512**		
***Hy-line* strain**			
Susceptible	Zero	14/20 = 0.7	6/20 = 0.3
Resistant	Zero	10/20 = 0.5	10/20 = 0.5
Fisher's Exact test	**0.3332**		
Fisher's Exact test	**0.2629**		

Table 4: Genotype frequency of *TLR4* - exon 2 with *EcoR1* restriction enzyme in experiment I.

Chickens type	Genotype frequency		
	AA	BB	AB
***Fayoumi* breed**			
Susceptible	Zero	20/20=1.00	Zero
Resistant	Zero	20/20=1.00	Zero
***Hy-line* strain**			
Susceptible	Zero	18/20=0.9	2/20=0.1
Resistant	Zero	10/20=0.5	10/20=0.5
Fisher's Exact test	**0.0138**		
Fisher's Exact test	**1.855****		

** = indication of a significance of Fisher's exact test at ($P < 0.01$)

Table 5: Genotype frequency of *TLR4* - exon 1 with *Taq1* restriction enzyme in experiment II.

Chickens type	Genotype frequency		
	AA %	BB %	AB %
***Fayoumi* breed**			
High immune response	8/20=0.4	Zero	12/20=0.6
Low immune response	2/20=0.1	10/20=0.50	8/20=0.4
Fisher's Exact test	**3.782****		
***Hy-line* strain**			
High immune response	Zero	8/20=0.4	12/20=0.6
Low immune response	6/20 =0.3	8/20=0.4	6/20 =0.3
Fisher's Exact test	**0.0151**		
Fisher's Exact test	**0.3008**		

** = indication of a significance of **Fisher's Exact** test at ($P < 0.01$)

Table 6: Comparison of genotype Frequencies of *TLR4* - exon 1 in resistant, susceptible, high immune and low immune response birds of *Fayoumi* breed and *Hy-line* strain using *Taq1* restriction enzyme.

Genotype	***Fayomi* Breed**				***Hy-line* Strain**			
	Susce-ptible	Resist-ante	High immune	Low immune	Susce-ptible	Resist-ante	High immune	Low immune
AA	zero	zero	0.4	0.1	Zero	zero	zero	0.3
AB	0.6	0.5	0.6	0.4	0.3	0.5	0.6	0.3
BB	0.4	0.5	zero	0.5	0.7	0.5	0.4	0.4

Table 7: Comparison of genotype frequencies of *TLR4*-exon 2 in susceptible, resistant, high immune and low immune response birds of the *Fayoumi* breed and *Hy-line* strain using *EcoR1* restriction enzyme.

Geno-types	***Fayoumi* breed**				***Hy-line* strain**			
	Susce-ptible	Resis-tant	High immune	Low immune	Susce-ptible	Resistant	High immune	Low immune
AA	zero	Zero	zero	zero	Zero	zero	zero	zero
AB	zero	Zero	zero	zero	0.1	0.5	zero	zero
BB	1.00	1.00	1.00	1.00	0.9	0.5	1.00	1.00

4. DISCUSSION

Genetic variations in *TLR4* gene affect the susceptibility and resistance to diseases. The association of *TLR4* gene and resistance against natural infection with *salmonella enteritidis* are reported in many breeds of chicken. In addition, SNPs of *TLR4* is observed in a chicken and cow (Leveque et al. 2003).

In the present study, the analysis of PCR-RFLP using *Taq1* restriction enzyme of *TLR4*-exon 1 (596 bp) revealed three genotypes in both *Fayoumi* breed and *Hy-line* strain. The results of experiment I (Table 4) showed that the genotype frequency of BB in infected (susceptible and resistant) chicken is equal to 0.4 and 0.5. This means that the BB genotype is more resistance to *salmonella* infection in the *Fayoumi* breed. But AB genotype frequency in infected (susceptible and resistant) *Hy-line* strain is equal to 0.3 and 0.5. The AB genotype is more in resistant *Hy-line* strain. An equal frequency of AB (0.5) and BB (0.5) genotype was observed in resistant birds in both *Fayoumi* breed and *Hy-line* strain. In susceptible birds, higher frequency of BB genotype (0.7) was observed in *Hy-line* strain and could use as a marker genotype for susceptibility to infection. However, a near frequency of AB (0.6) and BB (0.4) genotype was observed in *Fayoumi* breed and this result may be due to the *Fayoumi* breed is highly conserved as it not undergoes selection as in other commercial breeds (Pinard-vander Laan et al. 1998). So, it did not show variation in genotype as in case of *Hy-line* strain.

The results showed that by using *Taq1* restriction enzyme in *TLR4*-exon 1, the AB genotype is more frequent (0.6) in a high immune response chicks than AA and BB (0.4 and 0.0) in the *Fayoumi* breed. Moreover, the AB genotype gave a high immune response (0.6) than AA and BB genotypes (0.0 and 0.4) in *Hy-line* strain this result indicates that the AB genotypes produce a high immune response and can be used as a genetic marker to increase the immune response in chicks under study (Table 5).

In the present study, *TLR4*-exon 2 as shown in table (4) revealed only one genotype (BB) among *Fayoumi* breed by using the *EcoRI* restriction enzyme. However, two different genotypes were observed in *Hy-line* strain among susceptible birds BB with 0.9 and AB with 0.1 frequencies. In resistant birds, the gene frequency of BB and AB were (0.5). As the frequency of BB genotypes is more common in susceptible birds, this means that BB genotypes are more in susceptible than the AB genotypes and can be used as a marker-assisted selection for culling of susceptible birds.

The result of experiment II on *TLR4*-exon 2 (Table 7) showed only one genotype (BB) in both *Fayoumi* breed and *Hy-line* strain (high and low immune response birds). Liu et al. (2011) studied polymorphisms of chicken *TLR4*, they found genetic variations in exon 2 in 14 chicken breeds. Tibetan chicken and Red jungle fowl (characterized by a high resistance to salmonella infection) and these two breeds are highly conserved which had only BB genotype. While, the others breeds presented three genotypes of AA, BB, and AB. Ulupi et al. (2013) identified three genotypes (AA, AG and GG) in *TLR4*-exon 2 and recorded that resistant to salmonella enteritidis natural infection in the kampung chicken in all genotypes.

In conclusion, the *TLR4* genotype is related to the susceptibility and resistance to *salmonella* infection. The *Fayoumi* breed is a pure Egyptian conserved breed with one genotype (BB) for *TLR4*-exon 2 in resistant and susceptible birds compared with *Hy-line* strain chickens, which have variable genotypes (AB and BB), where (BB) frequency was higher in susceptible birds. So, we could not consider *EcoRI*-

TLR4-exon 2 as a marker for susceptibility or resistance to salmonella infection in *Fayoumi* breed. However, it may be used in *Hy-line* strain for detection the susceptible birds through BB genotype. Furthermore, *Taq1-TLR4*-exon 1 can be used as a marker for culling susceptible birds to salmonella infection in *Hy-line* strain through BB genotype, while AA genotype can be used as a marker-assisted selection for the culling of low immune birds in *Hy-line* strain.

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