Biotechnology in Animal Husbandry 27 (1), p 1-16 , 2011
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.58
DOI: 10.2298/BAH1101001R

CHARACTERIZATION OF TWO EGYPTIAN NATIVE CHICKEN BREEDS USING GENETIC AND IMMUNOLOGICAL PARAMETERS

H.A.I. Ramadan[1, 2], A. Galal[3], M.M. Fathi[3], S.A. El Fiky[1], H.A. Yakoub[1]

[1]Cell Biology Department, National Research Center, Dokki, Giza, Egypt
[2]Dept. of Biological Sciences, Faculty of Science, King Abdul Aziz University, P. O. Box 80203-, Jeddah 21589, Kingdom of Saudi Arabia
[3]Poultry Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
Corresponding author: Dr. Hassan A. I. Ramadan, e-mail: hameguid@yahoo.com
Original scientific paper

Abstract: In order to identify and characterize our native chicken breeds we used two approaches, one of them is genetic and the other concerns with the immunological status of the chickens. In this study, the first 539 bases of the mtDNA D-loop region of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) were amplified and sequenced. The alignment results showed an approximate tandem repeat of 60-base units with the first 34 nucleotides being exact. These 34-base units were completely identical and conserved in both Egyptian and GenBank database samples. The multiple alignment results showed also that there are three transversions specific for the Egyptian breeds only. Two of them are not specific for certain breed since both of the two breeds showed the normal and mutated nucleotide. The third transversions seems to be specific to Egyptian Dandarawi breed only. The first mutation site is at the position (457) where Adenine nucleotide transversed to Cytosine nucleotide. The second mutation site is at the position (464) where Guanine nucleotide transversed to Thymine nucleotide. The third mutation site is at the position (483) where Adenine nucleotide transversed to Thymine nucleotide. With respect to immunological parameters, it could be speculated that the Fayoumi strain had hyper responders to phytohemagglutinin-P (PHA-P) injection compared to Dandarawi ones. Opposite trend was noticed for anti-SRBCs antibody response.The results may give an insight into the genetic differentiation and immunological status of the Egyptian domestic fowl. The results did not show direct relation between the two approaches used. However, the results of the two approaches can complete the identification and characterization of the chickens. Also, they could be used in future as bases for more studies.

Key words: mitochondrial D-loop, immunological parameters, Egyptian chickens

Introduction

Fayoumi and Dandarawi breeds are pure Egyptian native chickens. These native breeds have the carcass characteristics and flavor desired by Egyptian consumers. They demonstrate better general disease resistance than imported breeds because they have evolved through natural selection for a long period in the prevailing environment. They showed a strong inherent scavenging and nested habit, less prone to predator attacks and can survive under harsh nutritional and environmental conditions. The Egyptian Fayoumi breed seemed to have more resistance to viral disease than other breeds of chicken *(Hoffmann, 2005)*. Fayoumi has been introduced in countries such as Tanzania *(Katule, 1989)*, Ethoepia *(Swan, 1996)* and Bangladesh *(Jensen, 1996)*. It was reported that the Sonali birds (male Rhode Island Red x female Fayoumi) reared under the semi-scavenging system in Bangladesh had a higher infection rate with Newcastle disease virus compared with indigenous and Fayoumi birds *(Biswas et al., 2005)*. So it was important to study the immunological status of our chickens in conjunction with other parameter, the genetic ones. Genetic diversity in a population is expected to enhance the chance for survival of the species. Mitochondrial DNA (mtDNA) is an available molecular tool for investigating evolutionary relationships and genetic variations within and between species because of its more rapid variability than nuclear DNA *(Avise et al., 1987; Zhang and Shi, 1992)*. The mitochondrial DNA (mtDNA) control region (CR), the major noncoding region of the animal mtDNA molecule, has a role in the replication and transcription of mtDNA molecules *(Clayton 1984, 1992)*. The central domain of the CR, containing the heavy strand's origin of replication, is relatively conserved *(Saccone et al., 1991)*. In contrast, the two domains that flank the central domain (domain I and domain III) are typically hyper-variable in base substitutions *(Saccone et al., 1987)*. Due to the fast rate of evolution of domain I and domain III, the CR has been typically deemed to be most appropriate for intra-specific studies *(Wenink et al., 1994)*. For genetic diversity studies, it is also important to identify the positions of nucleotide polymorphism where individuals have differences in their sequences. In this study, the first 539 bases of the mtDNA D-loop region (containing the first variable domain I) of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) and were amplified and sequenced and the polymorphic bases were recorded.

Materials and Methods

Birds and management. This experiment was carried out at poultry breeding farm, poultry production department, Faculty of Agriculture, Ain Shams University, Egypt. A total of 230 chicks (150 Fayoumi and 80 Dandarawi) were used. Chicks were wing-banded and brooded in electrical brooding batteries from

hatching up to 4 weeks of age, at when they were transferred to rearing batteries. All chicks were brooded and reared under similar environmental, managerial and hygienic conditions. The feed and water were provided ad libitum. They were fed a diet containing 18% crude protein and 2850 kcal ME/kg.

Amplification of the D-loop fragments and sequencing. Wing vein blood samples were obtained from eight live birds (four chickens per breed) without harming them; DNA was extracted using PURE Gene^{TM} DNA Purification Kit. As recommended by the manufacturer.

The conserved primer pair, L16750 (forward; 5'-AGG ACT ACG GCT TGA AAA GC-3') and H 547 (reverse; 5'- ATG TGC CTG ACC GAG GAA CAA G-3') were used to amplify the first 539 base fragment of the D-loop region of the birds. The primer number refers to the positions of the 3' end of the primer in the reference sequence *(Desjardins and Morais, 1990)*. The amplification reaction was carried out in a 25 µl reaction mixture consisting of 1.25 unit Taq polymerase (DyNAzyme), 1X enzyme buffer (1X is 10 mM Tris-HCl, pH 8.8 at 25 ^{0}C, 1.5 mM $MgCl_2$, 50 mM KCl and 0.1% Triton X-100) supplied by the manufacture, 1 µM of each forward and reverse primer, 0.2 mM dNTPs and 50 ng of DNA. The reaction mixture was overlaid with sterile mineral oil and was run in an MJ research PTC-100 Thermocycler. The PCR cycle profile was 94 ^{0}C for 2 min before the first cycle, then 94 ^{0}C for 1 min, 63 ^{0}C for 1 min and 72 ^{0}C for 1 min for 35 cycles. After the last cycle, the PCR mixture was incubated for a further 5 min at 72 ^{0}C. The reaction products (5 µl each) were used for electrophoresis with an appropriate size marker on 1.5% agarose in 1X-Tris acetate buffer (TAE). After electrophoresis the gels were stained with ethidium bromide and were examined with UV lamp at a wave length 312 nm to verify amplification of the D-loop fragment. The PCR products were purified using QIAquick PCR purification kit (Qiagen, Inc.) and the resulting purified products were used in the subsequent sequencing reactions. Sequencing was performed on an Applied Biosystems 310 genetic analyzer (Applied Biosystem) using BigDye terminator cycle sequencing ready reaction mixture according to manufacturer's instructions (Applied Biosystems). Direct submissions were made to GenBank database using BankIt. Sequence analysis and alignment were carried out using NCBI-BLASTN 2.2.5 version *(Altschul et al, 1997)* and Clustalw (1.82) multiple sequence alignment programs *(Thomson et al. 1994)*. The Clustalw program was also used for computing the alignment and the phylogenetic tree. The default parameters program was used. The phylogenetic tree was asserted by the bootstrapping technique.

Immunological parameters. Phytohemagglutinin injection (*In vivo* cell-mediated immunity assay). Response induced *in vivo* by mitogen was evaluated by injection of phytohemagglutinin-P (PHA-P) into the two-web between the second and the third digits of male chicks. Ten male chickens from each genetic line at 6 weeks of age were used. Each male was intradermally injected in the toe-

web of the left foot with 100 µg phytohemagglutinin-P (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 ml of sterile saline. The swelling response was measured with a constant tension caliper before injection and at 24, 48 and 72 hr after PHA-P injection. The toe-web swelling was calculated as the difference between the thickness of the toe-web before and after injection.

Sheep red blood cells (SRBCs). At 2 weeks of age, 30 chicks per strain were randomly assigned for assessing humoral immunity response. The sheep red blood cells (SRBCs) were collected and washed 3 times in phosphate–buffer saline (PBS). After that, the packed cells were brought to a 7% vol/vol solution in the PBS. At 2 wks of age, chicks were injected into thigh muscle with SRBC (3% suspension in PBS, 1 ml/chick) followed by a booster injection of SRBC suspension at 4 wks (after 14 days of the first injection). Blood samples were drawn at 7, 14 days from first and second injection. Plasma was stared at –20°C until tested. The antibody levels against SRBC were measured by hemagglutination test using 2% SRBCs suspension. Plasma was heat inactivated at 56°C for 30 min and then analyzed for total, mercaptoethanol-sensitive (Presumably IgM) and mercaptoethanol-resistant (IgG) anti-SRBC antibodies as previously described *(Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994)*. Briefly, 50 µL of plasma was added in an equal amount of PBS in the first column of a 96-well V-shaped bottom plate, and the solution was incubated for 30 min at 37°C. A serial dilution was then made and 50 µl of 2% SRBC suspension was added to each well. Total antibody titers were then read after 30 min of incubation at 37°C. The well immediately preceding a well with a distinct SRBC button was considered as the endpoint titer for agglutination. For MER (IgG) response, 50 µl of 0.01 M mercaptoethanol in PBS was used instead of PBS alone, followed by the previous mentioned procedure. The difference between the total and IgG response was considered to be equal to the IgM antibody level.

Relative weight of lymphoid organs. After completion of PHA-P assay, the same males (10 per genetic line) were weighed and slaughtered. The bursa of Fabricius, spleen and thymus (all lobes from left side of the neck) were removed and weighed to the nearest milligram.

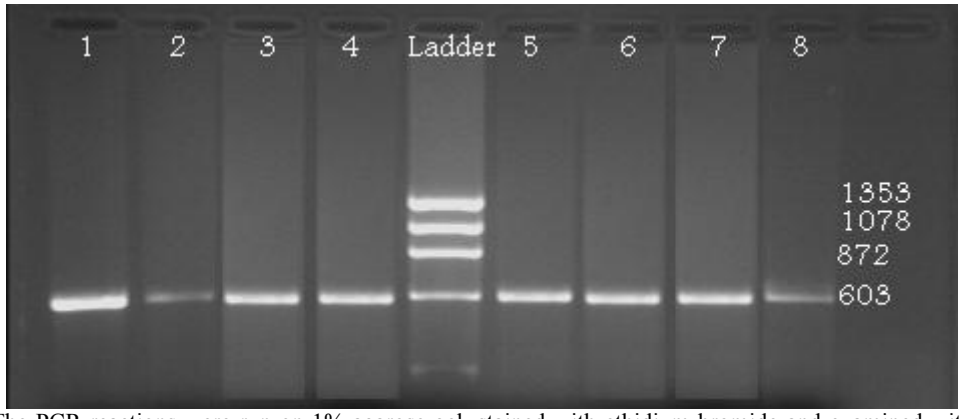
Statistical analysis

Data were subjected to a one-way analysis of variance with genetic group effect using the General Linear Model (GLM) procedure of SAS User's Guide, 2001.

Results and Discussion

Genetic diversity. In this study, the first 539 bases of the mtDNA D-loop region of two Egyptian native breeds (Fayoumi and Dandarawi, from El-Fayoum research station) were amplified using polymerase chain reaction. The reaction

products were run on 1% agarose gel and each of them gave only one sharp band in the correct size (Figure 1). Samples from the two breeds were sequenced in both directions (forward and reverse). The sequence of the fragments was corrected using Blast software. The final sequence results were corrected manually and submitted directly to GenBank database under the following accession numbers: (Fayoumi 1: EF 586879, Fayoumi 2: EF 586880, Dandarawi 1: EF 586881, Dandarawi 2: EF 586882, Dandarawi 3: EU352856). The data is available over the network data servers. The multiple sequence alignment results between these two breeds and the published results in GenBank database (Figure 2) showed an approximate tandem repeat of 60-base units with the first 34 nucleotides being exact (caagtcacctaactatgaatggttacaggacata). These 34-base units were completely identical and conserved in both Egyptian and GenBank database samples. It also contains in its center the published invariant tetradecamer (aactatgaatggtt) sequence. So we can conclude that the invariant sequence is composed of 34-base unit at the beginning of the approximate tandem repeat of 60-base unit. This agrees with the published tandem duplication of 60-base units which contains in its center the invariant tetradecamer (aactatgaatggtt) sequence in the member of the genus Gallus *(Akishinonomiya et al., 1994)*.



The PCR reactions were run on 1% agarose gel, stained with ethidium bromide and examined with UV. Samples from 1 to 4 for Dandarawi breed and samples from 5 to 8 for Fayoumi breed. The *Hae* III digest of Φ X174 DNA was used as ladder (1353, 1078, 872, and 603 base pairs).

Figure1. PCR amplification of chicken mitochondrial D loop fragment.

```
                                        First copy
EgyFay1EF586879
AGCTCCAAACCACTAACAAGTCACCTAACTATGAATGGTTACAGGACATAAATCTCACTC
AY704707
AGCTCCAAACCACTACCAAGTCACCTAACTATGAATGGTTACAGGACATAAATCTCACTC
```

```
AP003318
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
EgyDand3EU352856
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
DQ629875
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
AY644968
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
EF570457
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
AP003320
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
AB007750
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
EgyDand1EF586881
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
EgyFay2EF586880
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC
EgyDand2EF586882
AGCTCCAAACCACTAC CAAGTCACCTAACTATGAATGGTTACAGGACATA AATCTCACTC

**************↑**********************************************
                                        Second copy
EgyFay1EF586879
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
AY704707
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
AP003318
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
EgyDand3EU352856
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
DQ629875
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
AY644968
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
EF570457
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
AP003320
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
AB007750
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
EgyDand1EF586881
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
EgyFay2EF586880
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT
EgyDand2EF586882
TCATGTTCTTCCCCCAA CAAGTCACCTAACTATGAATGGTTACAGGACATA CATTTAACT

************************************************************

EgyFay1EF586879
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
AY704707
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
AP003318
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
EgyDand3EU352856
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
```

```
DQ629875
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
AY644968
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
EF570457
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
AP003320
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
AB007750
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
EgyDand1EF586881
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
EgyFay2EF586880
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC
EgyDand2EF586882
ACCATGTTCTAACCCATTTGGTTATGCTCGCCGTATCAGATGGATTTATTGATCGTCCAC

************************************************************

EgyFay1EF586879
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AY704707
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AP003318
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
EgyDand3EU352856
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
DQ629875
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AY644968
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AF512272
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AP003320
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
AB007750
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
EgyDand1EF586881
CTCACGAGAGATCAGCAACCCCTGCTTGTAATGTACTTCATGTCCAGTCTCAGGCCCATT
EgyFay2EF586880
CTCACGAGAGATCAGCCACCCCTTCTTGTAATGTACTTCATGACCAGTCTCAGGCCCATT
EgyDand2EF586882
CTCACGAGAGATCAGCCACCCCTTCTTGTAATGTACTTCATGTCCAGTCTCAGGCCCATT

***************↑******↑******************↑*****************
```

The first 34-base units in the approximate tandem repeat of 60-base units were underlined. The arrows show the positions of mutation sites in the Egyptian samples. The left column shows the breeds with their accession numbers and the asterisks indicate nucleotide identity between all samples.

Figure 2. Selected part of CLUSTALW (1.82) multiple sequence alignment between Fayoumi, Dandarawi and GenBank samples.

The results showed also that there are three SNP sites (transversions) revealed by the Egyptian chicken breeds. Two of them are specific for the Egyptian

breeds however they are not specific for certain breed since both of the two breeds showed the normal and mutated nucleotide. The third SNP site shows that it may be specific to Dandarawi breed only. The nucleotide numbers in Egyptian GenBank database samples were used to identify the mutation sites in all chicken samples. The first mutation site is at the position (457) where Adenine nucleotide transversed to Cytosine nucleotide. The second mutation site is at the position (464) where Guanine nucleotide transversed to Thymine nucleotide. The third mutation site is at the position (483) where Adenine nucleotide transversed to Thymine nucleotide. Other expected mutation site was noticed in only one bird (Fayoumi 1) where (C-A, at position 276). Mitochondria produce most of our cells' ATP, and thus their function is critical for our wellbeing. Over 100 proteins are involved in oxidative phosphorylation. Most of these proteins are encoded by nuclear genes, but 13 are encoded by mitochondrial genes. Furthermore, 22 tRNA genes and 2 rRNA genes are also encoded in the mitochondrial genome. In addition, more than 50 different disease-causing mitochondrial SNPs (single nucleotide polymorphism) have been identified in mammals, and this number is expected to increase as we become more proficient at detecting SNPs. Genetic diversity in the population enhances the chance for survival of the species. In conclusion, the sequence results Of the D-loop fragments identified the positions of nucleotide polymorphism where individuals have differences in their sequences. The results showed that there are three expected mutation sites (transversions) revealed by some of the Egyptian breeds only. Two of them are not specific for Dandarawi or Fayoumi since both of the two breeds showed the normal and mutated nucleotide while the third one may be specific for Dandarawi breed only.

Phylogenetic analysis. From the multiple alignment, the sequence differences can be summarized as indicated in Table 1. It can be easily noticed that the sequence of Egyptian Dandarawi No.3 is identical to the sequences in the Database. The most diverged pairs are DQ629875 (we consider it as out-group) and Egyptian Dandarawi No.2, where the number of differences is five. The phylogenetic tree based on the alignment is shown in Figure 3. It can be seen that the Egyptian groups are differentiated from the other ones, except for Egyptian Dandarawi No.3, which is identical to the other Database sequences. The Egyptian clade was also asserted when we ran the bootstrapping option of the ClustalW using the default bootstrapping parameters. The results gave an insight into the genetic diversity and divergence of the Egyptian domestic chickens; which might benefited through their evolution.

Table 1. The Polymorphic sites and their positions

Breed & Accession number	Variable sites and their positions					
	23	35	276	457	464	483
EgyDand1 EF586881	T	A	C	A	G	T
EgyDand2 EF586882	T	A	C	C	T	T
EgyDand3 EU352856	T	A	C	A	G	A
EgyFay1 EF586879	T	A	A	A	G	A
EgyFay2 EF586880	T	A	C	C	T	A
DQ629875	A	*	C	A	G	A
Database public sequence	T	A	C	A	G	A

The nucleotide positions were given with respect to the Egyptian nucleotide numbers in GenBank database. The left column shows the breeds with their accession numbers.

Lymphoproliferative response to PHA-P. Phytohemagglutinin-P, a T-cell mitogen, induces proliferation in T-lymphocytes. Injection of PHA-P at a selected site in chickens can be considered as an inducer of localized *in vivo* T-lyphoproliferative response *(Cheema et al., 2003)*. The results of swelling response measured at 24, 48 and 72h post-injection of PHA-P are presented in Table (2). With respect to cautenous basophilic hypersensitivity (CBH) it could be speculated that the Fayoumi strain exhibited significantly higher swelling response than that of Dandarawi ones at 24, 48 and 72h after PHA-P injection. The difference between strains for response to PHA-P injection could be attributed to the lymphoblastogenic response to PHA-P is presumed to be polygenic *(Morrow and Abplanalp, 1981)*. Also, T-cell mediated immune response of chicken has significant variation among birds of different genetic lineage *(Lamont and Smyth, 1984; Cheng and Lamont, 1988)*. Successful divergent selection of chickens for various T-cell functions suggests that many of these functions are highly heritable, and are often negatively correlated with body weight *(Yamamoto and Okada, 1990; Afraz et al., 1994)*.

Table 2. Means (±SE) of Phytohemagglutinin-P mediated (PHA-P) swelling in the toe-webs for Fayoumi and Dandarawi strains at 6 weeks of age

	Strain		
Time	**Fayoumi**	**Dandarawi**	**Probability**
24 h	0.203±0.02	0.188±0.03	0.01
48 h	0.111±0.02	0.103±0.03	0.01
72 h	0.083±0.02	0.073±0.03	0.05

24 h = toe-web swelling at 24 hr post PHA-P injection.
48 h = toe-web swelling at 48 hr post PHA-P injection.
72 h = toe-web swelling at 72 hr post PHA-P injection.

Body weight and lymphoid organs weight. Six weeks live body weight and relative lymphoid organs weight of Fayoumi and Dandarawi strains are presented in Table (3). There was no significant difference between strains for body weight. However, the Fayoumi strain had significantly higher relative bursa weight compared to Dandarawi ones. The bursa of Fabricius is a key lymphoid organ that is responsible for the development and maturation of B-lymphocytes, and the humoral antibody response is dependent on this central organ *(Zhang et al., 2006 and Cheema et al., 2007)*. For, example, a high antibody response to SRBC has been associated with a larger bursa size in White Leghorn chicken strains *(Ubosi et al., 1985)*. Furthermore, *Zhang et al. (2006)* showed a clear association between non-MHC genes and changes in the size of lymphoid organs by using highly inbred parental and recombinant congenic chicken lines.

The spleen provides microenvironment, which is needed for antigens presentation and concentrating them in the white pulps where T and B cell interactions, leads to the formation of antibodies *(White et al., 1975; Williams et al., 1991)*. Results indicated that the Fayoumi strain significantly has increased relative weight of spleen compared to Dandarawi ones. The immunological function of thymus is to provide a specific environment essential for T-cells differentiation, which is essential for cell-mediated immunity and modulation of immune response *(Owen, 1977)*. The differentiation is through subpopulation of thymus cells including T-helper, T-cytotoxic and T-suppressor cells. The present result indicated that the Fayoumi strain had significantly higher relative thymus weight compared to Dandarawi ones.

Table 3. Means (±SE) of live body weight and percentages of lymphoid organs relative to live body weight for Fayoumi and Dandarawi breeds at 6 weeks of age

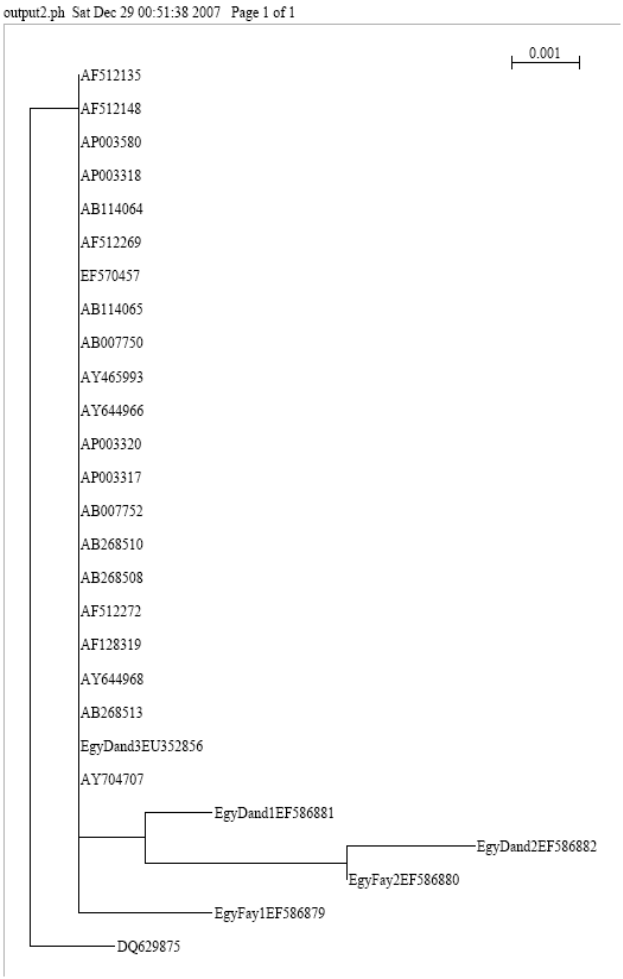
	Strain		
Trait	**Fayoumi**	**Dandarawi**	**Probability**
Body weight, g	457.50±13.99	429.13±15.71	NS
Bursa weight, %	0.292±0.03	0.302±0.04	0.01
Spleen weight, %	0.305±0.03	0.245±0.03	0.02
Thymus weight, %	0.371±0.02	0.334±0.03	0.05

Table 4. Means (±SE) of total antibody, IgG and IgM levels for Fayoumi and Dandarawi strains at 6 weeks of age

	Time			
Strain	**Day 7PPI**	**Day 14PPI**	**Day 7PSI**	**Day 14PSI**
	Total antibody level			
Fayoumi	3.80±0.03	3.40±0.02	5.33±0.02	3.60±0.03
Dandarawi	4.20±0.02	4.00±0.01	4.80±0.02	4.10±0.01
Probability	0.01	0.01	0.01	0.01
	Immunoglobulin-G (IgG)			
Fayoumi	2.20±0.02	1.80±0.03	2.11±0.01	2.10±0.01
Dandarawi	2.60±0.01	2.20±0.01	2.30±0.02	2.00±0.01
Probability	0.01	0.01	NS	NS
	Immunoglobulin-M (IgM)			
Fayoumi	1.60±0.03	1.60±0.01	3.22±0.02	1.50±0.03
Dandarawi	1.60±0.01	1.80±0.02	2.50±0.02	2.10±0.01
Probability	NS	NS	0.01	0.01

7PPI = 7 days post primary SRBCs injection
14PPI = 14 days post primary SRBCs injection
7PSI = 7 days post secondary SRBCs injection
14PSI = 14 days post secondary SRBCs injection

Anti-SRBCs antibody titer. Sheep red blood cells (SRBCs) have been chosen in this study as antigen because they are natural multi–determinant, non-pathogenic antigen and chicken phagocytosis of SRBCs opsonied with FC receptor for lysis and stimulate T-cell dependant response *(Saxena et al., 1997)*. Data illustrated in Table 4 showed that the Dandarawi breed had a significantly higher total anti-SRBC antibody titer at 7 and 14 days post primary SRBC-injection compared to Fayoumi ones. Conversely, the Fayoumi breed had significantly higher total anti-SRBCs antibody titer at 7 days post secondary SRBCs injection. Similar trend have not been observed at 14 days post secondary SRBCs injection.



Sample DQ629875 was used as out-group for its high diversity.

Figure 3. Phylogenetic tree constructed between the Egyptian and GenBank database chicken samples

The IgG anti-SRBC antibody titer measured at 7 and 14 days post primary injection of Dandarawi strain was significantly higher than that of Fayoumi ones. Inversely, there was no significant difference between breeds for IgG anti-SRBC antibody measured at 7 and 14 days post secondary SRBCs injection. *Okada and Yamamoto (1987)* demonstrated that the high IgG level was associated with high antibody response to SRBC and lipopolysaccharides. Also,

Martin et al. (1989) reported that IgG level was higher for high antibody level than low antibody level. With respect to IgM anti-SRBC antibody titer, it could be noticed that there was no significant difference between breeds for primary immune response. However, the Fayoumi breed had significantly higher IgM concentration measured at 7 days post secondary SRBC-injection compared to Dandarawi ones. Opposite trend was noticed 14 days post secondary SRBC-injection.

Conclusion

These results may give an insight into the genetic differentiation and immunological status of the Egyptian domestic fowl. These results did not show direct relation between the two approaches used. However, the results of the two approaches can complete the identification and characterization of the Egyptian native chicken breeds. Also, they could be used in future as bases for more studies.

Acknowledgment

The authors thank Mohamed Abouelhoda for his help in constructing the phylogenetic tree and helpful comments concerning the bioinformatics part.

Karakterizacija dve egipatske autohtone rase pilića korišćenjem genetskih i imunoloških parametara

H.A.I. Ramadan, A. Galal, M.M. Fathi, S.A. El Fiky, H.A. Yakoub

Rezime

U cilju identifikovanja i karakterizacije naših autohtonih rasa pilića, koristili smo dva pristupa, genetski i pristup koji se odnosi na imunološki status pilića. U ovom ispitivanju, prvih 539 baza mtDNK regiona D-petlje kod dve egipatske autohtone rase (Fayoumi i Dandarawi, iz El-Fayoum istraživačke stanice) je amplifikovano i sekvencirano. Rezultati poravnjanja pokazuju približno ponavljanje tandema od 60 baznih jednica sa prvih 34 nukleotida koji su tačni. Ovih 34 baznih jednica su bile u potpunosti identične i konzervirane u Egipatskoj banci gena i banci gena gde se čuvaju uzorci.
Višestruki rezultati poravnjanja pokazuju takođe da postoje tri transverzije koje su specifične samo za egipatske rase. Dve od njih nisu specifične za određenu rasu jer

obe rase su pokazale normalne i mutirane nukleotide. Treća transverzija se čini da je specifična samo za egipatsku Dandarawi rasu.

Prva lokacija mutacije je na poziciji (457) gde se adenin nukleotid transverzuje u cistin nukleotid. Drugo mesto mutacije je na poziciji (464) gde se guanin nukleotid transverzuje u timin nukleotid. Treće mesto mutacije je na poziciji (483) gde se adenin nukleotid transverzuje u timin nukleotid.

U vezi sa imunološkim parametrima, može se pretpostaviti da Fayoumi soj ima hiper odgovor/reakciju na fitoheamgglutinin-P (PHA-P) injekcije u poređenju sa Dandarawi sojem. Suprotni trend je registrovan kod anti-SRBCs reakcije antitela. Rezultati mogu dati uvid u genetsku diferencijaciju i imunološki status dve egipatske autohtnone rase. Rezultati nisu pokazali direktnu vezu između dva pristupa koji su korišćeni. Međutim, rezultati dobijeni korišćenjem dva pristupa mogu kompletirati identifikaciju i karakterizaciju pilića. Takođe, mogu se koristiti u budućnosti kao osnova za dalja istraživanja.

References

AFRAZ F., YAMAMOTO Y., OKADA I. (1994): Divergent selection for delayed-type wattle reaction of domestic fowls to BCC antigen. British Poultry Science, 35, 47-58.

AKISHINONOMIYA F., MIYAKE T., SUMI S., TAKADA M., OHNO S., KONDO N. (1994) : One subspecies of the red junglefowl (Gallus gallus gallus) suffies as the matriarchic ancestor of all domestic breeds. Proceedings of the National Academy Science, USA 91, 12505-12509.

ALTSCHUL S.F., STEPHEN F., MADEN T.L., SCHAFFER A.A., ZHANG J., ZHANG Z., MILLER W., LIPMAN D.J. (1997): Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25, 3389-3402.

AVISE J.C., ARNOLD J., BALL R.M., BERMINGHAM E., LAMB T., NEIGEL J.E., REEB C.A., SAUNDERS N.C. (1987) : Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics, 18, 489.

BISWAS P.K., BISWAS D., AHMED S., RAHMAN A., DEBNATH N.C. (2005): A longitudemic disease affecting semi-scavaning chickens reared under the partucatory livestock development project areas in Bangladesh. Avian Pathology, 34, 303-312.

CHEEMA M.A., QURESHI M.A., HAVENSTEIN G.B. (2003): A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. Poultry Science, 82, 1519-1529.

CHEEMA M.A., QURESHI M.A., HAVENSTEIN G.B., FERKET P.R., NESTOR K.E. (2007): A comparison of the immune response of 2003 commercial turkeys and a 1966 randombred strain when fed representative 2003 and 1966 turkey diets. Poultry Science, 86, 241-248.

CHENG S., LAMONT, S. J. (1988): Genetic analysis of immunocompetence measures in a white Leghorn chicken line. Poultry Science, 67, 989-995.

CLAYTON D.A. (1992): Transcription and replication of animal mitochondrial DNAs. In: WOLSTENHOLME D.R., JEON K.W. (eds), Mitochondrial genomes. International review of cytology, vol. 141. Academic Press, San Diego, CA, 217-232.

CLAYTON D.A. (1984): Transcription of the mammalian mitochondrial genome. Annual Review of Biochemistry, 53, 573-594.

DESJARDINS P., MORAIS R. (1990): Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. Journal of Molecular Evolution, 212, 599-634.

HOFFMANN I. (2005): Research and investment in poultry genetic resources-challenges and options for sustainable use. World's Poultry Science Journal, 61, 57-69.

JENSEN A.H. (1996): Semi-scavenging model for rural poultry holding. In: Proceeding 20th World Poultry Congress, New Delhi, India, 1-5 September, Vol. I, 61-70.

KATULE A. (1989): Studies on prospects of improving the performance of the local chicken population in Tanzania by cross breeding. Ph.D. Thesis, Sokoine University of Agriculture, Morogoro, United Republic of Tanzania.

LAMONT S.J., SMYTH J.R. (1984): Effect of selection for delayed amelanosis on immune response in chickens. 2. Cell-mediated immunity. Poultry Science, 63, 440-442.

MARTIN A., GROSS W.B., SIEGEL P.B. (1989): IgG and IgM responses in high and low antibody selected lines of chickens. Journal Heredity, 80, 249-252.

MORROW, P.R., ABPLANALP, H. (1981): Genetic control of T-lymphocyte mitogenesis in chickens. Immunogenetics, 13, 189-200.

OKADA I., YAMAMOTO Y. (1987): Immunocompetence and Marek's disease resistance in three pairs of chicken lines selected for different immunological characters. Poultry Science, 66, 769-773.

OWEN J.J.T. (1977): Ontogenesis of lymphocytes. In: B and T all in immune recognition. John wile and Sons. New York. NY, 21-34.

QURESHI M.A., HAVENSTEIN G.B. (1994): A comparison of the immune performance of a 1991 commercial broiler with a 1957 randombred strain when fed "typical" 1957 and 1991 broiler diets. Poultry Science, 73,1805-1812.

SACCONE C., ATTIMONELLI M., SBISA E. (1987): Structural elements highly preserved during the evolution of the D-loop containing regions in vertebrate mitochondrial DNA. Journal of Molecular Evolution, 26, 205-211.

SACCONE C., PESOLE G., SBISA E. (1991): The main regulatory region of mammalian mitochondrial DNA: structure-function model and evolutionary pattern. Journal of Molecular Evolution, 33, 83-91.
SAS INSTITUTE. (2001) SAS/STAT User's Guide: Statistics. Ver. 8.2. SAS Institute Inc. Cary. NC.
SAXENA V.K., SINGH H., PAI S.K., KUMAR S. (1997): Genetic studies on primary antibody response to sheep erythrocytes in guinea fowl. British Poultry Science, 38:156-158.
SWAN S.E.J. (1996): Training in rural poultry development. Terminal report, project TCP/ETH/4455. Rome, FAO.
THOMPSON J.D., HIGGINS D.G., GIBSON T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22, 4673-4680.
UBOSI C.O., GROSS W.B., HAMILTON P.B., EHRICH M., SIEGEL P.B. (1985): Aflatoxin effects in White Leghorn chickens selected for response to erythrocyte antigen.2. Serological and organ characteristics. Poultry Science, 64, 1071-1076.
WENINK P.W., BAKER A.J., TILANUS M.G.J. (1994): Mitochondrial control region sequences in two shorebird species, the Turnstone and the Dunlin, and their utility in population genetic studies. Molecular of Biology Evolution, 11, 22-31.
WHITE R.G., HENDERSON D.C., ELDAMI M.B., NIELSON S.A. (1975): Localization of a protein antigen in the chicken spleen. Effect of various manipulative procedures on the morphogenesis of the germinal center. Immunology, 28, 1-21.
WILLIAMS W.J., BEULTER E., ERSLEV A.J., LITCHMAN M.A. (1991) Neutrophils, eosinophils and basophils. I: Ersler and M.A. Litchman (ES.). McGraw-Hi II Publishing Company, 760-780.
YAMAMOTO Y., GLICK B. (1982): A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of Fabricius. Poultry Science, 61, 2129-2132.
YAMAMOTO Y., OKADA I. (1990): Two-way selection for survival time of allograft in chickens. Japanese Poultry Science, 27, 337-337.
ZHANG Y.P., SHI L.M. (1992) Mitochondrial DNA polymorphism in animals: A review. Zoology Research, 13:289.
ZHANG H.M., HUNT H.D., KULKARNI G.B., PALMQUIST D.E., BACON L.D. (2006): Lymphoid organ size varies among inbred lines 63 and 72 and their thirteen recombinant congenic strains of chickens with the same major histocompatability complex. Poultry Science, 85, 844-853.

Received 13 August 2010; accepted for publication 16 March 2011