GENETIC DIVERSITY AT TLR7 GENE IN NIGERIAN INDIGENOUS CHICKENS AND ISA BROWN COMMERCIAL LAYER CHICKEN

[1]Ukwu, H. O., [*]Ibe, S. N., [*]Nwachukwu, E. N. & [+]Imumorin, I. G., [1]Audu, M. O.

[1] Department of Animal Breeding and Physiology, Federal University of Agriculture Makurdi, Benue State, Nigeria.

[*] Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria.

[+]School of Biological Science, Georgia Institute of Technology, Atlanta, USA.

Corresponding author's e-mail: ukwuhenry@gmail.com

ABSTRACT

The objective of this study was to estimate genetic diversity at TLR7 locus in the Nigerian indigenous chicken populations and ISA Brown commercial layer chicken. Eight (8) chicken populations (naked neck, normal and frizzled-feathered Nigerian indigenous chickens in rain forest and Guinea Savannah regions respectively, Fulani ecotype chicken and ISA Brown commercial layer chicken) were used for this study. Blood samples were collected from each of the eight chicken populations. Genomic DNA was isolated from each blood sample using the Zymo Quick-gDNA^{TM} Miniprep kit. Sequencing of chTLR7 gene was done using the Sanger Sequencing Chemistry. Genetic distance estimates ranged from 0.007 to 0.054 and were close to zero, which suggests that the chickens have a close ancestor. The estimates of nearest-neighbour statistic ranged from 0.227 to 0.714, which showed that the chicken populations were part of the same panmictic population, hence were not genetically differentiated (P>0.05) at the TLR7 locus.

Keywords: Chicken, diversity, genetic distance, nucleotide, Toll-like receptor.

INTRODUCTION

Toll-like receptor 7 (TLR7) gene is one among the genes implicated in the immune system of vertebrates, especially in recognition of viral Pathogens. TLR genes are proven associated with resistance to infectious diseases. Chicken toll-like receptor (chTLR) genes have been reported to be polymorphic among different avian breeds, suggesting a varied resistance across numerous chicken breeds (Ruan *et al*., 2012a; 2012b). Although TLR domains are highly conserved among species and among different members of the multi-gene family because of their crucial participation in signaling transduction (Barreiro *et al*., 2009), recent reports have revealed that mutation on their sequences can bring about single nucleotide polymorphisms (SNPs), hence creating genetic variants among populations of the same species. For example, four new single nucleotide polymorphisms (SNPs) of TLR7 gene were discovered in Chinese ducks (Zhu *et al*., 2011). Recent reports suggest that the ability of certain individuals to respond properly to TLR ligands may be impaired by single nucleotide polymorphisms (SNPs) within TLR genes, resulting in an altered susceptibility to, and development of infectious or inflammatory disease (Schroder and Schumann, 2005).

However, TLR7 gene has not been investigated in the Nigerian indigenous chicken populations. The Nigerian indigenous chickens found in different ecological locations in Nigeria may have evolved adaptive characteristics in response to changes in natural environmental conditions, which could potentially affect individual's ability to sense invading viral pathogen-associated molecular patterns.

We hypothesize that indigenous chicken ecotypes in different regions of Nigeria may have developed varied ability to respond to viral pathogens.

This study, therefore, was designed to access the genetic diversity at the TLR7 locus in the Nigerian indigenous chickens and ISA Brown commercial layer chicken.

MATERIALS AND METHODS

Blood sample collection

About 1 ml of blood sample was aseptically collected from the brachial vein of each bird, separately into vacutainer tubes (SARSTEDT Monovette®) containing Ethylene-diamine-tetra-acetic acid (EDTA), using sterile needles. Blood samples were collected from naked neck, normal-feathered, frizzle-feathered

indigenous chickens from rainforest zone, Guinea Savannah zone, Fulani ecotype chicken, and ISA Brown commercial layers chickens.

DNA extraction, Polymerase Chain Reaction and Sequencing

Genomic DNA was isolated from each blood sample using the Zymo Quick-gDNA™ Miniprep kit (D3024, Zymo Research Corporation, Irvine, CA, USA) following the manufacturer's instructions. The concentration and purity of each DNA sample was ascertained using Nanodrop Spectrophotometer.

Three (3) DNA samples from each genetic group were taken for polymerase chain reaction (PCR) amplification of TLR7 gene. The primers used were designed using the Primer3 and BLAST option at the NCBI database (www.ncbi.nlm.nih.gov). The following primers were used: Forward-AGGCTTGCTGTTGTGGCATGGA and Reverse- ACTGGAAGCCCTTCCTCCACTGT. PCR were performed in a 50 µl reaction volume containing 10 µl of 5X FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia), 2.5 µl each of forward and reverse primers, 31 µl of nuclease-free water and 4 µl sample DNA template. PCR conditions consist of 1 cycle of 95°C for 4 minutes initial denaturation, 35 cycles each of 95°C for 30 seconds denaturation, 62°C for 30 seconds annealing, 72°C for 1 minute elongation, followed by 72°C for 10 minutes final elongation. DNA sequencing of chTLR7 gene was done using the Sanger Sequencing Chemistry. Laboratory analysis was performed at African Bioscience Laboratory in Ibadan, Nigeria.

RESULTS AND DISCUSSION

Estimates of Nearest-neighbour statistic (Snn) between the Nigerian indigenous chickens in rain forest region, Guinea savannah region, Fulani ecotype and ISA Brown layer chickens ranged from 0.292 to 0.714, with a value of 0.227 for the sequences pulled together (Table 1). Permutation test on estimates of Snn with 1000 replications and excluding sites with alignment gaps gave *P*-values ranging from 0.186 to 0.687 and were not significant ($P>0.05$). This implies that the Nigerian indigenous chickens from rain forest and Guinea savannah, Fulani ecotype and ISA Brown layer chickens were not genetically differentiated at the TLR7 locus.

The nearest-neighbour statistic (Snn) is a measure of how often the nearest neighbours (in sequence space) of sequences are from the same locality in geographic space (Hudson, 2000). The Snn estimates are expected to be near one when the populations at the two localities are highly differentiated and near one-half when the populations at the two localities are part of the same panmictic population (Hudson, 2000). The nearest–neighbour statistic is very useful when genomic data are collected on individuals from two or more geographic locations. In this study, analysis of TLR7 gene sequences from the Nigerian indigenous chicken populations and ISA Brown commercial layer chicken revealed that the nearest-neighbour statistic (Snn) were close to one-half, which implies that the Nigerian indigenous chickens from rain forest, Guinea savannah, Fulani ecotype chicken and ISA Brown commercial layer chicken were part of the same panmictic population.

Table 1: Nearest-neighbour statistic, Snn between populations of Nigerian indigenous chickens and ISA Brown commercial layer chicken

Populations	S_{nn}	P-value of S_{nn}
Fulani ecotype VS. Guinea savannah	0.525	0.517^{NS}
Fulani ecotype VS. ISA Brown	0.467	0.382^{NS}
Fulani ecotype VS. Rain forest	0.714	0.186^{NS}
Guinea savannah VS. ISA Brown	0.292	0.854^{NS}
Guinea savannah VS. Rain forest	0.388	0.687^{NS}
ISA Brown VS. Rain forest	0.438	0.635^{NS}
Entire population	0.227	0.459^{NS}

NS= not significant (P>0.05)

The estimates of mean genetic distance among Nigerian indigenous chicken populations from different locations and ISA Brown commercial layer chicken are presented in Table 2, while Table 3 shows the estimates of mean genetic distance among the Nigerian indigenous chicken genotypes and ISA Brown

commercial layer chicken. The estimates of mean genetic distance (Table 2) ranged from 0.007 to 0.014, and were also close to zero. The least between group mean genetic distance (0.007) was observed between the Fulani ecotype chicken and the rain forest chicken, while the highest mean distance (0.014) was between ISA Brown layer chicken and Guinea savannah chicken. Analysis of TLR7 gene sequences from the different chicken genotypes (naked neck, frizzle-feathered and normal feathered chickens) from different regions, Fulani ecotype chicken of Nigeria and ISA Brown commercial layer chicken revealed that genetic distance estimates ranged from 0.006 to 0.054 and were also close to zero (Table 3). The observed close-to-zero estimates of genetic distance is an indication that the Nigerian indigenous chickens genotypes found in different regions of the country and ISA Brown commercial layer chicken were closely related at the TLR7 locus. This report seems to be the first investigation of TLR7 locus in the Nigerian indigenous chickens and ISA Brown commercial layer chicken. The level of genetic relatedness observed in this study showed that although the indigenous chickens are found in different region in Nigeria, the locus was not under ecological or geographical divergence; consequently, the TLR7 locus is highly conserved among the Nigerian indigenous chicken populations. This finding corroborates the report of Bulumulla *et al*. (2011) that the TLR7 locus is a conserved region among Sri Lankan indigenous chicken population and Ceylon jungle fowl (*Gallus lafayetti*).

Table 2: Estimates of mean genetic distance among Nigerian indigenous chicken populations and ISA Brown commercial chicken

*Populations	FEC	GSC	ISA	RFC
FEC	-	0.002	0.003	0.002
GSC	0.011	-	0.003	0.003
ISA	0.010	0.014	-	0.003
RFC	0.007	0.013	0.010	-

Estimates of genetic distance are shown below the diagonal; Standard error of estimates are shown above the diagonal; FEC= Fulani ecotype chickens; GSC= Guinea Savannah chickens; ISA= ISA Brown layer chickens; RFC= Rain forest chickens

Table 3: Estimates of mean genetic distance among the Nigerian indigenous chicken genetic groups and ISA Brown commercial layer chicken

Groups	FE	GF	Gn	GN	ISA	RF	Rn	RN
FE	-	0.004	0.006	0.003	0.004	0.004	0.0040	0.003
GF	0.014	-	0.007	0.004	0.005	0.003	0.004	0.004
Gn	0.042	0.054	-	0.007	0.006	0.007	0.006	0.007
GN	0.007	0.012	0.048	-	0.004	0.003	0.004	0.005
ISA	0.026	0.034	0.045	0.028	-	0.005	0.004	0.005
RF	0.010	0.009	0.051	0.006	0.030	-	0.004	0.005
Rn	0.020	0.022	0.045	0.019	0.030	0.016	-	0.005
RN	0.007	0.014	0.047	0.012	0.031	0.013	0.024	-

Estimates of genetic distance are shown below the diagonal; Standard error of estimates are shown above the diagonal; FE= Fulani ecotype chicken, GN= Guinea savanna naked neck chicken, Gn= Guinea savannah normal chicken, GF= Guinea savannah frizzle-feathered chicken, RF= Rain forest frizzle-feathered chicken, RN= Rain forest naked neck chicken, Rn= Rain forest normal chicken, ISA= ISA Brown commercial layer chicken

The percent identity of TLR7 gene sequence from the Red jungle fowl (*Gallus gallus*) retrieved from the National Centre for Biotechnology information (NCBI) database (Accession No.NC_006088.5), with the consensus sequence of TLR7 gene of Nigerian indigenous chickens and ISA brown was 99%. The estimates of genetic distance between the Red jungle fowl and the Nigerian indigenous chickens and ISA

brown commercial layer chicken at the TLR7 locus were very low and ranged from 0.007±0.002 to 0.012±0.003 (Table 4). This implies a very close relationship and low level of divergence at the TLR7 locus in the Nigerian indigenous chicken populations, ISA Brown commercial layer chicken and the Red jungle fowl. This also suggests that the Nigerian indigenous chickens and ISA Brown commercial layer chicken are not genetically distanced from the Red jungle fowl at the TLR7 locus. This is in line with the report of Bulumulla *et al*. (2011) that TLR7 locus is a conserved region among the indigenous chicken population.

Table 4: Genetic distance between the Nigerian indigenous chickens, ISA Brown commercial layer chicken and the Red jungle fowl (reference sequence)

Chickens	Red jungle fowl
Fulani ecotype	0.008±0.004
Guinea savannah	0.012±0.003
Rain forest	0.007±0.002
ISA Brown layers	0.008±0.003

CONCLUSION

The results of this study revealed that genetic distance estimates among the Nigerian indigenous chickens, ISA Brown commercial layer chickens and the Red jungle fowl were close to zero, which suggests that the chickens were not under ecological divergence. The estimates of nearest-neighbour statistic obtained in this study showed that the Nigerian indigenous chickens from the rainforest, Guinea savannah, Fulani ecotype and ISA Brown commercial layer chickens were not genetically differentiated at the TLR7 locus, thus were part of the same panmictic population.

REFERENCES

Barreiro, L. B., Ben-Ali, M., Quach, H. (2009). Evolutionary dynamics of human Toll-like receptors and their different contributions to host defense. *PLoS Genet*., 5(7): e1000562. https://doi.org/10.1371/journal.pgen.1000562

Bulumulla, P. B. A. I. K., Silva P. and Jianlin, H. (2011). Genetic Diversity at Toll Like Receptor 7 (TLR7) Gene of Sri Lankan Indigenous Chicken and Ceylon Jungle Fowl (*Gallus lafayetti*). *Tropical Agricultural Research* 22 (4): 367 – 373

Hudson, R.R. (2000). A new statistic for detecting genetic differentiation. *Genetics*, 155: 2011 – 2014.

Ruan, W. K., Wu, Y. H., An, J. Cui, D. F., Li, H. R. and Zheng, S. J. (2012a). Toll-like receptor 2 type-1 and type-2 polymorphisms in different chicken breeds. *Poult. Sci*., 91: 101 – 106

Ruan, W. K., Wu, Y. H., An, J. and Zheng, S. J. (2012b). Polymorphisms of chicken Toll-like receptors 4, 15 and 21 in different breeds. *Poult. Sci*., 91: 2512 – 2516.

Schroder, N. W. and Schumann, R. R. (2005). Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. *Lancet Infect. Dis*., 5: 156 – 164.

Zhu, W., Li, H., Song, W., Hu, Y., Zhu, C., Song, C., Shu, J. and Chen, K. (2011). Four new single nucleotide polymorphisms (SNPs) of Toll-like receptor 7 gene discovered in Chinese ducks. *African J. Biotechnol*., 10(83): 19294 – 19298