Genetic Diversity and Expression of the Chicken Toll-like Receptor 7 Gene in the Nigerian Unimproved Native and Isa Brown Commercial Layer Chickens

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Abstract

The chicken toll-like receptor 7 (chTLR7) gene is a member of a multigene family that is responsible for recognition of pathogens in vertebrates. The toll-like receptor gene family has been investigated in different breeds of chicken, however, there is dearth of information on the diversity of the chTLR7 gene in the Nigerian native chicken population. This work was aimed at investigating the genetic diversity at the chTLR7 gene and its expression in the Nigerian unimproved native and ISA Brown commercial layer chickens. The chicken population used in this study were naked neck, frizzle and normal-feathered native chickens from Guinea savannah and rain forest regions, the Fulani ecotype chickens and ISA Brown commercial layer chickens. The Sanger sequencing approach was used to sequence the chTLR7 gene of DNA samples isolated from each of the chicken population. Total RNA was extracted from the thymus and the liver tissues from two chickens from each of the populations studied. Complementary DNA (cDNA) was synthesized from total RNA for qPCR assay using SensiFASTTM cDNA preparation kit. The expression of cTLR7 gene was evaluated using SYBR Green qPCR reaction, with β-actin as the reference gene. The estimates of nucleotide diversity, gene flow, genetic distance and nearest-neighbour statistics were in the ranges of 0 to 0.019, -0.096 to 0.400, 0.007 to 0.054 and 0.227 to 0.714, respectively. These suggest that the native chickens and ISA Brown layer chicken were not genetically differentiated at the chTLR7 gene, hence could belong to the same panmictic population. However, expression of the chTLR7 gene in the thymus and liver differed significantly ($P<0.01$) among the chicken populations, with the rain forest naked neck native chicken showing the highest chTLR7 gene expression. However, ISA Brown commercial layer chicken had the lowest chTLR7 expression. The observed differences in chTLR7 gene expression suggests that the Nigerian unimproved native chickens could have more antiviral response than ISA Brown commercial layer chicken.

Keywords: Chicken, diversity, genetic distance, phylogeny, Toll-like receptor.
Running title: Genetic diversity of toll-like receptor 7 gene in the Nigerian native chickens

Diversité Génétique et Expression du Gène du Récepteur Toll-like 7 chez les Poules Nigérianes Non Améliorées et les Poules Commerciales ISA Brown

Résumé

Le gène du récepteur Toll-like 7 chez la poule (chTLR7) est un membre d'une famille multigénique responsable de la reconnaissance des pathogènes chez les vertébrés. La famille des gènes des récepteurs Toll-like a été étudiée dans différentes races de poules, cependant, il y a un manque d'informations sur la diversité du gène chTLR7 dans la population de poules indigènes nigérianes. Ce travail visait à étudier la diversité génétique du gène chTLR7 et son expression chez les poules indigènes nigérianes non améliorées et les poules commerciales ISA Brown. La population de poules utilisée dans cette étude comprenait des poules à cou nu, des poules frisées et des poules à plumes normales provenant des régions de savane guinéenne et de forêt tropicale, les poules écotypes Fulani et les poules commerciales ISA Brown. La méthode de séquençage Sanger a été utilisée pour séquencer le gène chTLR7 à partir d'échantillons d'ADN isolés de chaque population de poules. L'ARN total a été extrait des tissus du thymus et du foie de deux poules de chaque population étudiée. L'ADN complémentaire (ADNc) a été synthétisé à partir de l'ARN total pour le test qPCR en utilisant le kit de préparation SensiFAST™ ADNc. L'expression du gène cTLR7 a été évaluée à l'aide de la réaction qPCR avec SYBR Green, avec la β-actine comme gène de référence. Les estimations de la diversité des

nucléotides, du flux génétique, de la distance génétique et des statistiques des plus proches voisins étaient dans les plages de 0 à 0,019, -0,096 à 0,400, 0,007 à 0,054 et 0,227 à 0,714, respectivement. Cela suggère que les poules indigènes et les poules commerciales ISA Brown n'étaient pas génétiquement différenciées au niveau du gène chTLR7, et pourraient donc appartenir à la même population panmictique. Cependant, l'expression du gène chTLR7 dans le thymus et le foie différait de manière significative (P<0,01) entre les populations de poules, avec les poules à cou nu de la forêt tropicale montrant la plus haute expression du gène chTLR7. En revanche, les poules commerciales ISA Brown avaient la plus faible expression du gène chTLR7. Les différences observées dans l'expression du gène chTLR7 suggèrent que les poules indigènes nigérianes non améliorées pourraient avoir une réponse antivirale plus forte que les poules commerciales ISA Brown.

Mots-clés : Poule, diversité, distance génétique, phylogénie, récepteur Toll-like.

Introduction

Toll-like receptor genes are among the genes implicated in the immune system of vertebrates (Alcaide and Edwards, 2011). Farm animal species and humans have innate immune system with the primary function of detecting the presence of disease-causing organisms through the toll-like receptor (Duan *et al*., 2022). Toll-like receptors, also known as pattern-recognizing receptors (PRRs) can identify pathogen-specific molecules also known as pathogen-associated molecular patterns (PAMPs), which exist in microorganisms (Akira and Takeda, 2004). The role of toll-like receptors in the stimulations of proinflammatory signal transduction pathways as well as recognition and ingestion of microbial pathogens has been reported (Underhill and Ozinsky, 2002). In vertebrates, toll-like receptors are well-known for their capability in triggering adaptive immunity (Akaide and Edwards, 2011). Recent candidate gene investigations have revealed that toll-like receptors can recognize different molecules (Uematsu and Akira, 2008). In response to invasion by microbial molecular patterns which are not expressed by the host, TLRs activate the adaptive immunity of chickens by triggering the population of reactive oxygen and Nitrogen intermediates, as well as inflammatory cytokines (Kannaki *et al.*, 2010). Whenever various TLRs are activated, the innate immunity is also activated, and this therefore results to development of antigen-specific acquired immunity (Akira and Takeda, 2004).

The toll-like receptor multigene family comprises over ten genes with significant variations among vertebrates (Werling *et al*., 2009). Since TLR genes have been implicated to be linked with disease resistance, members of this multigene family can be investigated as markers in selection for disease resistance (Kannaki *et al*., 2010), as well as in genetic diversity studies of unimproved native chickens to exploit single nucleotide polymorphisms (SNPs) that may be used in selection programmes.

With recent advances in molecular genetics, TLRs and their corresponding genes in the domestic chicken (*Gallus gallus domesticus*) have been well characterized (Lynn *et al*., 2003; Smith *et al*., 2004; Fukui *et al.*, 2005; Iqbal *et al.*, 2005; Philbin *et al*., 2005;Yilmaz *et al*., 2005). Toll-like receptor 7 (TLR7) gene is among the multigene implicated in recognition of viral PAMPs. The chicken toll-like receptor 7 (chTLR7) gene encodes 1047 amino acid protein with 62 percent identity to human toll-like receptor 9 (Philbin *et al.*, 2005). Although the TLR domain are highly conserved (Barreiro *et al*., 2009), recent reports have revealed that mutation on their sequences can bring about single nucleotide polymorphisms, hence creating genetic variants among populations of the same species. For example, four new single nucleotide polymorphisms (SNPs) of the TLR7 gene were discovered in Chinese ducks (Zhu *et al.*, 2011). In addition, three genotypes were all found in each mutation site on TLR7 gene in Chinese ducks (Zhu *et al*., 2011). It has been documented that the presence of SNPs in TLR

genes could affect an individual's potential to respond to TLR ligands (Schroder and Schumann, 2005). It has also been reported that variation in disease resistance across different breeds of chicken could be attributed to polymorphisms existing in the chTLR genes (Ruan *et al.*, 2012a, 2012b). The expression of chTLR7 gene in lymphoid tissues of chickens has been reported (Raj *et al*., 2009; Ravi-Kumar *et al.*, 2010; Ramasamy *et al*., 2010; Kannaki *et al*., 2015). Breed differences in chTLR7 gene expression has also been reported (Abasht *et al*., 2009).

However, the chTLR7 gene has not been investigated in the Nigerian unimproved native chicken population. The Nigerian unimproved native chickens found in different ecological niche in Nigeria are known for their ability to survive harsh environmental challenges, but the mechanism behind their hardiness is not documented. The native chickens may have evolved adaptive characteristics at the expense of production genes in response to changes in natural environmental conditions, which may have potentially reshaped their abilities to spot invading pathogen-associated molecular patterns. Since the chTLR7 gene has been characterized (Bulumulla *et al*., 2011), there is need to perform genetic diversity study at the chTLR7 locus in the Nigerian unimproved native chicken populations as well as ISA Brown commercial layer chicken in Nigeria. We hypothesized that the unimproved native chicken ecotypes in guinea savannah and rain forest regions of Nigeria may have developed wide-ranging ability to respond to viral pathogens.

This study, therefore, was designed to determine the genetic diversity at the chTLR7 gene, and its expression in the liver and thymus tissues of the Nigerian unimproved native chickens and ISA Brown commercial layer chicken.

MATERIALS AND METHODS

Collection of blood samples

Blood samples (about 1 mL) were collected from the brachial vein of each bird, separately into vaccutainer tubes (SARSTEDT Monovette®) containing Ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant, using sterile needles. Blood samples were collected from eight chicken populations comprising naked neck, normal-feathered, frizzle-feathered native chickens from rainforest zone of South-East; naked neck, normal-feathered and frizzle-feathered native chickens from Guinea Savannah zone of North-Central; Fulani ecotype chickens from Fulani settlement in Taraba state, and ISA Brown commercial layers chickens.

DNA isolation and purity

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

Polymerase Chain Reaction (PCR) and sequencing of the chTLR7 gene

Three DNA samples from each chicken genetic group were taken for polymerase chain reaction (PCR) amplification of TLR7 gene. The primers used for PCR amplification of target gene (chTLR7) were designed using the Primer3 and BLAST option at the NCBI database (www.ncbi.nlm.nih.gov). The characteristics of the primers used are presented in Table 1. Polymerase chain reactions (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. FIREPol® Master Mix reagent composition includes FIREPol® DNA polymerase, 0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1 % w/v Tween-20, 12.5 mM $MgCl_2$, 1 mM dNTPs (200 µM each of dATP, dCTP, dGTP, dTTP), blue dye, yellow dye and compound that increases sample density for direct loading. 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. Sequencing of chTLR7 gene was performed with the same PCR primers using the Sanger Sequencing Chemistry.

Table 1: Primers and sequences for amplification of TLR7

Primer	Primer sequence	GC Content %
TLR7-F	AGGCTTGCTGTTGTGGCATGGA	54.55
TLR7-R	ACTGGAAGCCCTTCCTCCACTGT	56.52

Tissue collection

Two clinically healthy chickens from each of the eight genetic groups were slaughtered for tissue collection. The following tissues comprising the liver and the thymus were aseptically collected from each bird and were immediately transferred into separate 1.5 mL Eppendorf tubes containing 1 ml of RNALater solution. The two chickens from each genetic group served as biological replicates.

RNA Extraction

Total RNA was extracted separately from each of the thymus and the liver using ISOLATE II RNA Mini kit (BIOLINE, USA). The kit is highly efficient for purification of total RNA from RNALater treated tissues (and other samples) in as little as 30 minutes.

Complementary DNA (cDNA) synthesis

Complementary DNA (cDNA) was synthesized using SensiFAST^{TM} cDNA synthesis kit (BIOLINE, USA), following manufacturer's instructions. The kit provides a rapid and sensitive method for first strand cDNA synthesis that can be used in real-time PCR investigations.

Real-Time Polymerase Chain Reaction (qPCR)

The expression pattern of the chTLR7 RNA in the liver and thymus were determined by qPCR assay. The EVA Green dye chemistry was used in detection. The PCR primers were designed using Primer3 and blast option at the NCBI website. The information on primers used is presented in Table 2. House-keeping gene β-actin was used as the reference gene. Real-Time PCR reactions were done in triplicates in 25 µL reaction volume comprising 5 µL PCR Master mix (FIREPol^{R,} Solis BioDyne, Estonia), 0.6 µL each of forward and reverse primers, 4 µL of template cDNA and 14.8 µL of nuclease-free water in a smart cycler tube. A 25 µL non-template reaction mixture was also run alongside as a negative control. The qPCR conditions were as follows: 40 cycles each of initial denaturation at 95°C for 15 minutes; denaturation at 95°C for 15 s; annealing at 65°C for 20 s and extension at 72°C for 20 s.

Table 2: Primer information for qPCR of the chTLR7 RNA

Primer	Primer sequence	GC Content %	T_m (min/max)
TLR7-F	CCTCGAT CTCAACCCTACTTCT	50.00	62.67
TLR7-R	CAGTATCTTTTCCTCACCACACA	43.48	60.99

Assembling of qPCR data and statistical analysis

Data on qPCR of chTLR RNA were assembled using Microsoft Excel (2010). The average C_T values of technical replicates and change in C_T values (ΔC_T = C_T target gene – C_T reference gene) were also inferred using Microsoft Excel (2010). Fold changes in expression levels of the chTLR7 gene in the Nigerian unimproved native chickens were calculated relative to samples of ISA Brown layer chickens. Fold changes ($2^{-\Delta\Delta ct}$) in gene expression were determined using the comparative $\Delta\Delta C_T$ method as described by Livak and Schmittgen

(2001). Data on fold changes in gene expression were subjected to One-way Analysis of Variance (ANOVA) and means with significant differences were separated using Duncan's multiple range tests. ANOVA and mean separation were performed using Statistical Package for the Social Sciences (SPSS) software version 21.0.

Results and Discussion

Expression of chTLR7 gene in lymphoid tissues of the Nigerian unimproved native chickens and ISA Brown commercial layer chicken

The constitutive expression of chTLR7 gene in the liver and thymus tissues of the Nigerian unimproved native chickens relative to ISA Brown layer chickens is shown in Table 3. The expression of chTLR7 gene in the liver varied significantly ($P<0.01$) among the eight chicken genetic groups. The highest fold change (2.07±0.07) in chTLR7 gene expression was observed in the rain forest naked neck chicken (RN). However, expression of chTLR7 gene in the liver of rain forest frizzle-feathered chicken (RF), rain forest normal chicken (Rn), Guinea savannah naked neck chicken (GN), Guinea savannah frizzle-feathered chicken (GF), Guinea savannah normal chicken (Gn) and Fulani ecotype chicken (FE) did not vary significantly ($P>0.05$), but were higher than that of ISA Brown layer chicken.

The expression of chTLR7 gene in the thymus tissues varied significantly ($P<0.01$) among the eight chicken genetic groups. The chTLR7 gene expression in thymus of RN (2.18±0.04 fold), Rn (2.14±0.07 fold), GN (2.30±0.08 fold) and Gn (2.34±0.04 fold) were similar but significantly ($P<0.01$) higher than that of RF (1.39±0.02 fold), FE (1.30±0.02 fold) and GF (1.13±0.02 fold). The expression of chTLR7 gene in thymus of GF chicken was not significantly different ($P>0.01$) from that of ISA Brown layer chicken.

The chTLR7 is an immune-related gene specifically known for the recognition of viral PAMPs (Akira *et al.* 2006). It has been reported that Constitutive expressions of genes that are linked to the immune system vary among different breeds of chicken and consequently lead to variation in disease resistance among different breeds of chicken (Ruan et al. 2012). The constitutive expression of chTLR7 plays a vital role in the innate immune response of chickens through detection of invading viral pathogens. Our comparative analysis of chTLR7 gene expression in the liver and thymus of the Nigerian unimproved native chickens and ISA Brown commercial layer chicken showed that the Nigerian unimproved native chickens expressed higher chTLR7 gene than ISA Brown layer chickens in the lymphoid organs studied. This suggests that the Nigerian unimproved native chickens could have better genetic potential to resist viral invasion than ISA Brown commercial layer chickens. This observation is an indication of breed differences in chTLR7 gene expression which has been earlier reported (Abasht *et al.* 2009; Ramasamy *et al.* 2010).

Table 3: Relative expression of the chTLR7 gene in the liver and thymus tissues of the Nigerian unimproved native chickens and ISA Brown commercial layer chicken

*Tissues	----------Mean±SE fold change----------								
	RN	RF	Rn	GN	GF	Gn	FE	IS	P-value
Liver	2.07±0.07^{a}	1.34±0.02^{b}	1.47±0.05^{b}	1.39±0.02^{b}	1.49±0.03^{b}	1.37±0.05^{b}	1.52±0.05^{b}	1.01±0.14^{c}	0.000
Thymus	2.18±0.04^{a}	1.39±0.02^{b}	2.14±0.07^{a}	2.30±0.08^{a}	1.13±0.02^{bc}	2.34±0.04^{a}	1.30±0.02^{b}	1.01±0.10^{c}	0.000

*RN= Rain Forest naked neck chicken, RF= rain forest frizzle-feathered chicken, Rn= rain forest normal chicken,

GN= Guinea savannah naked neck, GF= Guinea savannah frizzle-feathered chicken, Gn= Guinea savannah normal chicken, FE= Fulani ecotype chicken, IS= ISA Brown commercial layer chicken

Estimates of Nearest-neighbour statistic, Snn between populations of Nigerian native chickens and ISA Brown commercial layer chickens

Estimates of Nearest-neighbour statistic (Snn) between the Nigerian unimproved native chickens and ISA Brown commercial layer chickens ranged from 0.292 to 0.714, with a value of 0.227 for the sequences pulled together (Table 4). 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 unimproved native chickens and ISA Brown commercial layer chickens were not genetically differentiated at the chTLR7 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 close to one when two populations at different locations are highly differentiated and near one-half when the populations at different locations are part of the same panmictic population (Hudson 2000). The nearest–neighbour statistic is particularly useful when genomic data are collected on individuals from two or more geographical locations. In this study, analysis of chTLR7 gene sequences from the Nigerian unimproved native chicken populations and ISA Brown commercial layer chicken revealed that the nearest-neighbour statistic (Snn) was close to one-half, which implies that the Nigerian unimproved native chickens and ISA Brown commercial layer chicken were part of the same panmictic population.

Table 4: Nearest-neighbour statistic, Snn between populations of Nigerian unimproved native chickens and ISA Brown commercial layer chickens

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)

Genetic distance and relationship of the chTLR7 gene sequences in Nigerian unimproved native chickens and ISA Brown commercial layer chickens

The estimates of mean genetic distance within the Nigerian unimproved native chicken populations from distinct locations and ISA Brown commercial layer chicken are presented in Table 5, while Table 6 shows the estimates of between groups mean genetic distance.

Estimates of mean genetic distance among the Nigerian native chicken genotypes and ISA Brown commercial layer chicken are shown in Table 7.

The results of this study revealed that estimates of mean genetic distance within the Fulani ecotype chicken, guinea savannah chickens, rain forest chickens and ISA Brown commercial layer chickens were 0.010±0.002, 0.019±0.004, 0.013±0.004 and 0.012±0.003 respectively (Table 5). Genetic distance estimates within each of the chicken population was close to zero, which implies that the chickens were very closely related.

The estimates of between group mean genetic distance (Table 6) 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 chTLR7 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 7). The observed close-to-zero estimates of genetic distance is an indication that the Nigerian native chickens' genotypes found in different regions of the country and ISA Brown commercial layer chicken were closely related at the chTLR7 locus.

This report is the first investigation of chTLR7 locus in the Nigerian native chickens and ISA Brown commercial layer chicken. There was no available literature on phylogenetic analysis of the chTLR7 gene in the Nigerian native chicken populations and ISA Brown commercial layer chickens. The level of genetic relatedness observed in the phylogenetic analysis showed that although the indigenous chickens are found in different region in Nigeria, the locus was not under ecologic or geographical divergence; consequently, the chTLR7 locus is highly conserved among the Nigerian native chicken populations. This finding corroborates the report of Bulumulla et al. (2011) that the chTLR7 locus is a conserved region among Sri Lankan indigenous chicken population and Ceylon jungle fowl (*Gallus lafayetti*).

Table 5: Estimates of mean genetic distance within the Nigerian unimproved native chicken populations and ISA Brown commercial layer chicken

Chicken populations	d±SE
Fulani ecotype chicken	0.010±0.002
Guinea savannah chicken	0.019±0.004
Rain forest chicken	0.013±0.004
ISA Brown layer chicken	0.012±0.003

d = estimate of genetic distance, SE= Standard error of estimate

Table 6: Estimates of mean genetic distance among the Nigerian unimproved native 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 7: Estimates of mean genetic distance among the Nigerian unimproved native chicken genotypes 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	0.004	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

Phylogenetic analysis of TLR7 gene in the Nigerian unimproved native chicken populations and ISA Brown commercial layer chicken

Evolutionary relationship of the chTLR7 gene in the Nigerian unimproved native chickens and ISA Brown layer chickens is shown in Figure 1. Phylogenetic analysis of the chTLR7 gene sequences revealed that the Nigerian unimproved native chickens from different regions, and ISA Brown commercial layer chickens were not distinguishable based on their geographic locations. Phylogenetic tree also revealed that ISA Brown commercial layer chicken, Fulani ecotype chicken and normal-feathered chickens from rain forest and Guinea savannah regions were genetically closer than the frizzle-feathered and naked neck chickens at the chTLR7 locus.

This report is the first investigation of the chTLR7 locus in the Nigerian unimproved native chickens and ISA Brown commercial layer chicken. There was no available literature on phylogenetic analysis of the chTLR7 gene in the Nigerian unimproved native chicken populations. The level of genetic relatedness observed in the phylogenetic analysis showed that although the indigenous chickens are found in different region in Nigeria, the locus was not under ecologic or geographical divergence; consequently, the chTLR7 locus is highly conserved among the Nigerian unimproved native chicken populations.

Evolutionary relationship of the chTLR7 gene in the Nigerian unimproved native chickens, ISA Brown commercial layer chicken and the chTLR7 gene sequence from the Red Jungle Fowl

In this study, 521-bp fragment of the chTLR7 gene sequences from the Nigerian native and ISA Brown commercial layer chickens were analyzed with TLR7 sequence from the red jungle fowl. The percent identity of the chTLR7 gene sequence from the red jungle fowl (*Gallus gallus;* Accession No: NC_006088.5) with the Consensus sequence of chTLR7 gene of the Nigerian unimproved native chickens and ISA Brown commercial layer chicken was 99%. The estimates of genetic distance between the red jungle fowl and the Nigerian unimproved native chickens and ISA Brown commercial layer chicken at the chTLR7 locus were low and ranged from 0.007±0.002 to 0.012±0.003 (Table 8). This implies a close relationship and low level of divergence at the chTLR7 locus in the Nigerian unimproved native chicken populations, ISA Brown commercial layer chicken and the red jungle fowl.

Phylogenetic analysis of chTLR7 gene sequences of the Nigerian unimproved native chicken populations, ISA Brown commercial layer chicken and the red jungle fowl (Figure 2) revealed close relationship at the chTLR7 locus. This implies that the Nigerian unimproved native chickens and ISA Brown commercial layer chicken are not genetically distanced from the red jungle fowl at the chTLR7 locus. This is in line with the report of Bulumulla *et al.* (2011) that chTLR7 locus is a conserved region among the indigenous chicken population.

Table 8: Genetic distance between the Nigerian native 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

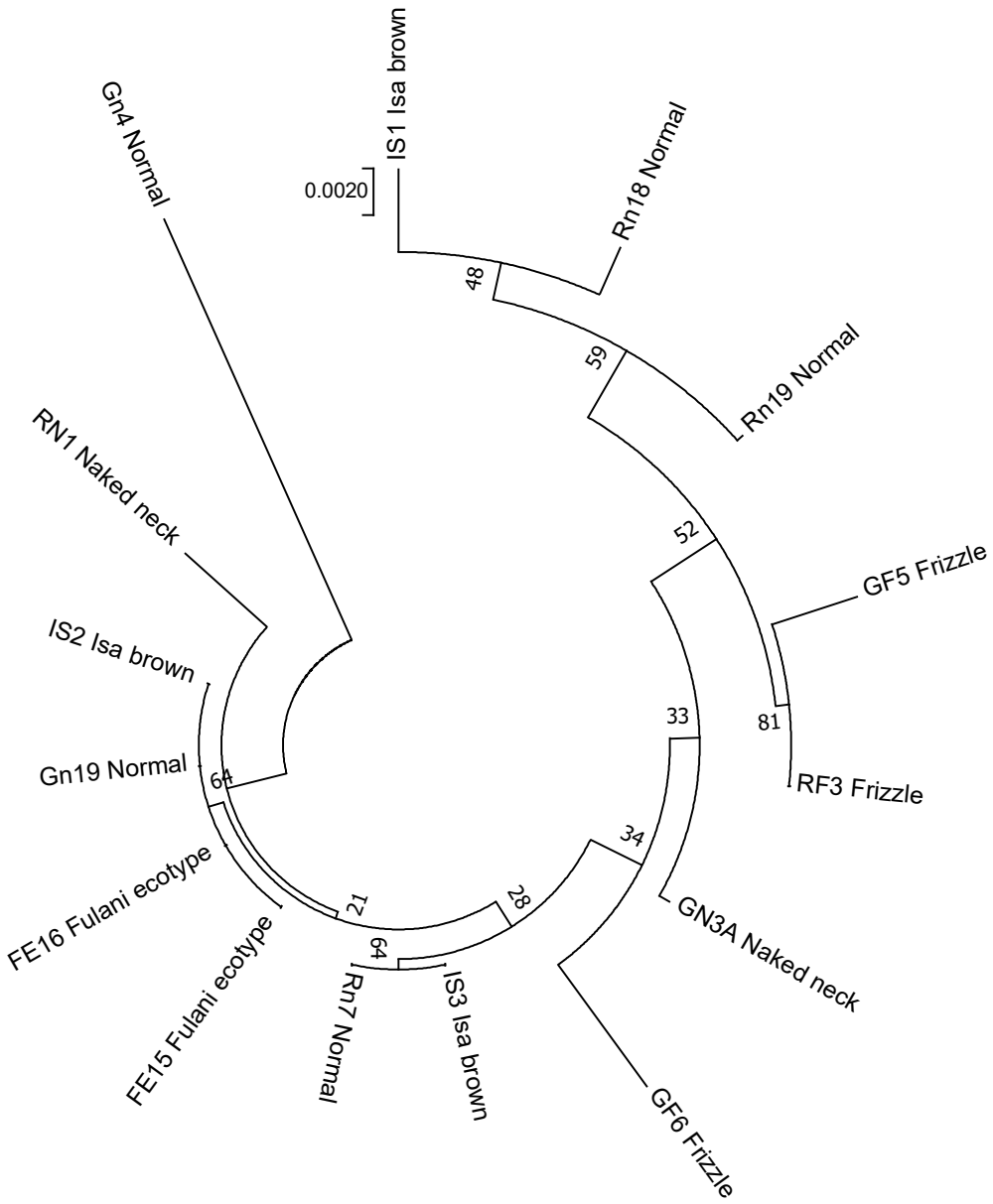

Figure 1: Phylogenetic (Neighbour-joining) tree constructed from TLR7 gene sequences from the Nigerian unimproved native chickens and ISA Brown commercial layer chicken

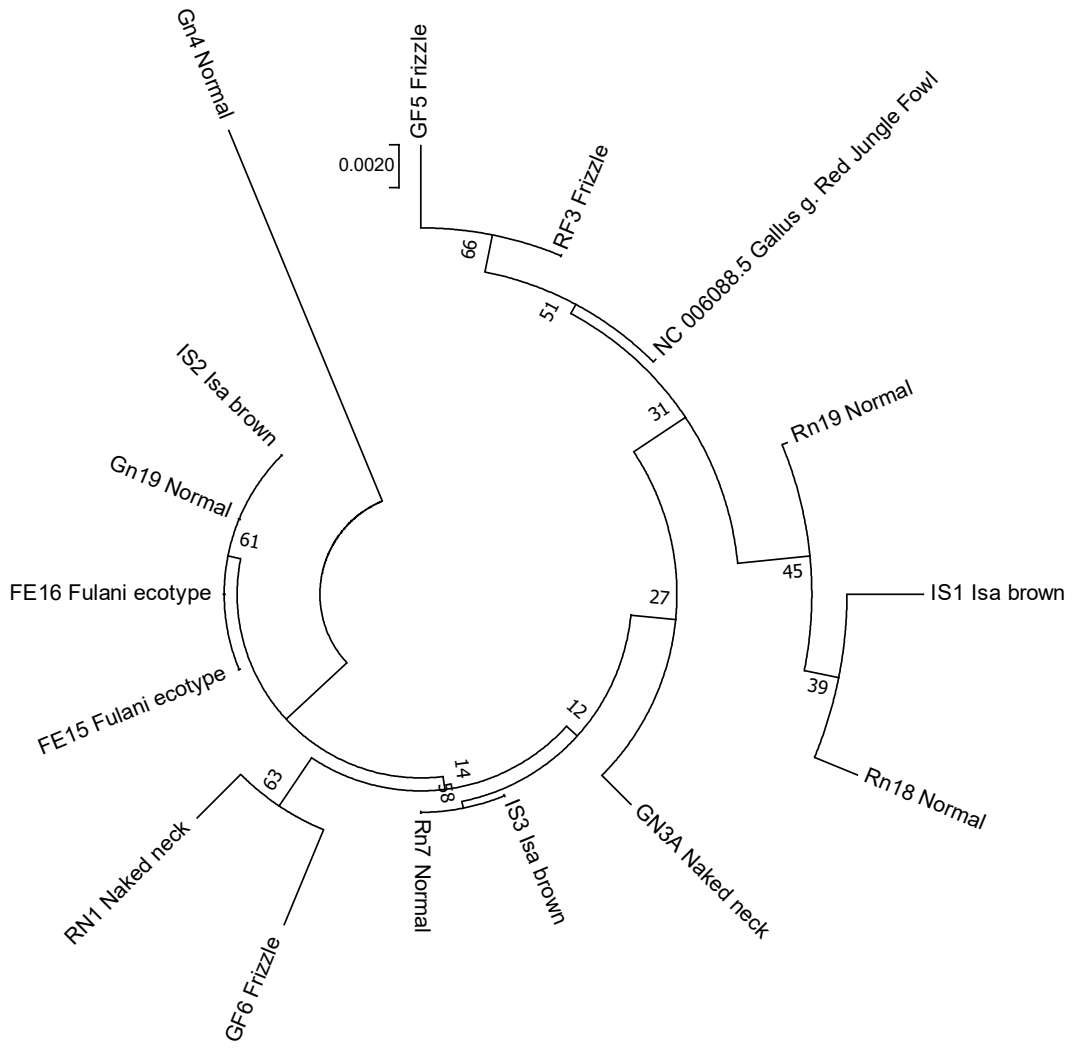

Figure 2: Phylogenetic (Neighbour-joining) tree constructed from the chTLR7 gene sequences of the Nigerian native chickens, ISA Brown commercial layer chickens, and the chTLR7 sequence belonging to the red jungle fowl.

Conclusion

It could be concluded from the results of this study that genetic distance estimates among the Nigerian native 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 native chickens from the rainforest, Guinea savannah, Fulani ecotype and ISA Brown commercial layer chickens were not genetically differentiated at the chTLR7 locus, thus were part of the same panmictic population.

Evolutionary analysis of the chTLR7 gene sequences of the Nigerian unimproved native chickens, ISA Brown and the Red jungle fowl showed close relationship at the chTLR7 locus, which implies that the chTLR7 locus is highly conserved among the chicken populations. Gene expression analysis of the chTLR7 mRNA also shows that the Nigerian indigenous chickens could have better genetic potential for antiviral immune response than ISA Brown commercial layer chicken.

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