Molecular Characterization of Coding Sequence and mRNA Expression Pattern of Toll-like Receptor 15 in Japanese Quail (Coturnix japonica) and Indigenous Chicken Breeds (Aseel and Kadaknath)

Kannaki T. Ramasamy¹, Premchandra Verma¹, Maddula R. Reddy² and Shanmugam Murugesan²

¹ Immunology section, Indian Veterinary Research Institute, Bareilly, 243122, U.P, India
² Project Directorate on Poultry, The Indian Council of Agricultural Research, Hyderabad, 500030, A.P, India

Toll-like receptors (TLRs) play a crucial role in innate immune response of mammalian and avian species. The chicken TLR repertoire consists of ten genes. TLR15 is avian specific TLR with no mammalian homologue. The present study has characterized full-length coding sequence of TLR15 in Japanese quail and Indian indigenous chicken (Aseel and Kadaknath). Open reading frame of all three sequences were 2,607 bp long encoding 868 amino acids similar to that of broiler chicken. Japanese quail, Aseel and Kadaknath sequences showed 95.1%, 99.7% and 99.8% similarity with broiler chicken TLR15 sequence at nucleotide level respectively. Domain architecture analysis revealed minor variations in number and position of leucine rich repeats (LRRs) in the ectodomain region of all three sequences. Phylogenetic analysis revealed TLR15 groups with high bootstrap support to avian TLR1 family members. TLR15 mRNA expression in a range of tissues as quantified by real time PCR was found significantly (P < 0.01) higher in Aseel in comparison to Kadaknath and Japanese quail in most of the tissues investigated.

Key words: Indigenous chicken, Innate immunity, Japanese quail, Toll-like receptor 15


Introduction

Innate immunity, once considered as non-specific immune response of meager role is now considered as a fundamental orcheister of overall immune response. Toll-like receptors (TLRs) are important members of innate immune system and members of TLR family have diversified functionally to recognize distinctive pathogen associated molecular patterns (PAMPs) such as peptidoglycan, LPS, flagellin, double stranded RNA etc. These receptors are highly conserved from insects to human and at least 11 TLRs have been identified in mammals so far (Anderson, 2000; Takeda and Akira, 2005). TLRs recognize PAMPs in an efficient and non self reactive manner to initiate pro-inflammatory mediators which finally culminate in the initiation of adaptive immune response (Janeway and Medzhitov, 2002; West et al., 2006).

To date, 10 TLRs have been identified in chicken (TLR 1 (types 1 and 2), TLR2 (types 1 and 2), TLR3, TLR4, TLR5, TLR7, TLR15, and TLR21) (Fukui et al., 2001; Iqbal et al., 2005; Philbin et al., 2005; Roach et al., 2005; Yilmaz et al., 2005; Higgs et al., 2006; Boyd et al., 2007; Kaiser, 2007; Keestra et al., 2007). Higgs et al. (2006) first identified and characterized the presence of TLR15, a novel, avian specific TLR, which has no mammalian counterpart. Till date, TLR15 sequence has been characterized only in chicken among the avian species.

Indigenous chicken breeds are considered to be more disease resistant than commercial layer and broiler breeds (Rout et al., 1992) and are also genetically distinct (Wimmers et al., 2000). Aseel breed of chicken is known for its hardiness and is raised for meat and fighting qualities whereas Kadaknath breed is known for their delicious black coloured meat. Both the breeds sexually mature approximately six weeks later than White Leghorn chicken (Biswas et al., 2010). Kadaknath has lower body weight at different ages, attains earlier sexual maturity and has higher egg production than Aseel (Haunshi et al., 2011). Quails apart from being good source of eggs and meat, are extensively used as a model for embryological and cancer research (Panda and Mohapatra, 1998). Further, they are sturdy and known for resistance to many infectious diseases including Ranikhet disease, ascariasis and coccidiosis (Mohapatra, 2002). However, the mechanism of innate resistance in these species remains to be elucidated. In the present study we sequenced and characterized TLR15 coding region of indigenous chicken...
breeds (Aseel and Kadaknath) and Japanese quail. The expression of TLR15 mRNA in a range of tissue has also been quantified by real time RT-PCR.

Materials and Methods

Experimental Birds

Japanese quails of thirty weeks old and cockerels of twenty four week old Indian indigenous chicken breeds (Aseel and Kadaknath) (each for n = 4) maintained under standard management conditions at the Department of Poultry science, College of Veterinary Science, Hyderabad and Project Directorate on Poultry, respectively, were used in the present study. All experiments were performed with the approval of the Institute Animal Ethics and Monitoring Committee. Birds were sacrificed by cervical dislocation and tissues such as liver, spleen, small intestine (SI), skin, bone marrow (BM) and bursa were collected aseptically from all individuals and processed separately.

RNA Extraction and cDNA Synthesis

Total RNA from different tissues was extracted by using TRI reagent (T9424, Sigma-Aldrich Co., St.Louis, MO, USA), following manufacturer’s instruction. To avoid the possible traces of genomic DNA, 5 µg of each RNA sample was incubated at 37°C for 10 min with 5 U of RNase free DNase, following this step DNase was inactivated by incubation at 65°C for 10 min. Subsequently, first strand cDNA was synthesized from 1 µg of total RNA using oligo (dT) primer and MuMLV reverse transcriptase (EP0351, MBI Fermentas Life Science, Ontario, Canada) in a 20 µl reaction mixture following the recommendations of manufacturer.

Coding Sequence Amplification and Sequencing of TLR15

To amplify the full-length open reading frame (ORF) of TLR15 cDNA of Japanese quail and indigenous chicken breeds, overlapping primer sets were designed based on publicly available broiler chicken TLR15 mRNA sequence (DQ267901; Higgins et al., 2006). The primer sets (Primer 1: F1, 5’-ATGAGGATCCTTATTGGGAG-3’; R1, 5’-GCTGTCAAGCTCTTCAATTAAG-3’; Primer 2: F2, 5’-TGACTTGTGAGACCCAGAT-3’; R2, 5’-TGAGCAGTTGGAACCT-3’; primer 3: F3, 5’-TACACCCATCGAAAGCCCTT-3’; R3, 5’-GATGGCGTTGTCGCTTAATGT-3’; primer 4: F4, 5’-ATCGAGGATGATACGATCT-3’; R4, 5’-TACAGTTCACTGACACCCA-3’; primer 5: F5, 5’-GGAACGTGAGTATAGCAGATA-3’; R5, 5’-TCATCCATCTCAATTTACCC-3’) were designed to amplify the fragments exactly covering full length ORF from spleen cDNA sample. The reaction conditions for amplification in PCR were same for all the fragments. The 50 µl PCR reaction contained 50 pmol of each forward and reverse primers, 1 µl template cDNA, 200 µM of dNTP mix, 1.0 mM MgCl2 and 2.5 U Taq DNA polymerase (EP0401, MBI Fermentas Life Science, Ontario, Canada) in 1X Taq buffer. Amplification conditions were as follows: an initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, followed by final extension at 72°C for 10 min. PCR amplicons verified by 1% agarose gel electrophoresis were purified and sequenced by using an automated DNA sequencer (ABI prism, model 377, version 3.0, Foster City, CA, USA).

Sequence Analysis

The sequences of the fragments were aligned using MegAlign of DNA star software (Lasergene®, Madison, WI, USA) and complete coding sequences of Aseel, Kadaknath and Japanese quail were identified and translated to amino acid sequences using EditSeq of same software. The signal peptide sequence was identified by SignalP program (www.cbs.dtu.dk/service/SignalP). The domain structure, LRRs and transmembrane region were identified by online softwares like SMART (Schultz et al., 1998; http://www.smart.embl-heidelberg.de/) and TMHMM (Letunic et al., 2004; http://www.cbs.dtu.dk/service/TMHMM/). Phylogenetic tree based on evolutionary distances was constructed by using MEGA 3.1 software (Kumar et al., 2004).

Quantification of TLR15 mRNA

The relative expression of TLR15 mRNA was quantified by real-time PCR by using Mx3000P™ system (Stratagene, La Jolla, CA, USA). The TLR15 gene specific primer set was designed from published sequence (DQ267901). F: 5’-TCTTCTGTCATCTGTTGTG-3’; R: 5’-CCTGGATTTGGGATCTTC-3’. β-actin gene was used as endogenous control. Primer set used was F: 5’-CAGCCACACTTCTAATGAG-3’; R: 5’-ACGACCAGGGCATACAGG-3’. All reactions were performed in duplicates in a total volume of 25 µl reaction containing 1X QuantiTect SYBR Green PCR master mix containing X QuantiTect SYBR Green PCR master mix (SYBR Green I dye, ROX passive reference dye, HotStarTaq DNA polymerase and dNTPs with dUTPs in optimized buffer, 204141, Qiagen GmbH, Hilden, Germany), 10 pmol of each primer and 0.5 µl of cDNA template. Thermal profile consisted of an initial denaturation at 94°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30s; annealing at 55°C for 30s and extension at 72°C for 30s. For each sample dissociation curve was generated after the completion of amplification and analyzed to confirm the specificity of amplicon. In each PCR reaction no template control was included to check contamination of master mix. Non-reverse transcribed RNA (10 ng) of each sample was used instead of cDNA to check contamination of samples with genomic DNA, failure of amplification confirms the purity of sample. To assess the efficiency of primers, standard curves for each primer pair were generated using serially diluted transcribed RNA sample. PCR efficiency was calculated from the slope of standard curves. The resulting threshold cycle (Ct, a fractional PCR cycle number at which the change in reporter dye (D RN) passes the significant threshold) values were normalized to the endogenous control, β-actin (ΔCt = Ct value of target gene-Ct value of β-actin). To convey the inverse relationship between starting template concentration and Ct value, results were expressed and conveyed the inverse relationship between starting template concentration and Ct value, results were expressed and

Ramasamy et al.: TLR15 in Native Chicken and Quail 169
analyzed as 40-ΔCt values, interpreted as higher 40-ΔCt value implying greater gene expression (MacKinnon et al., 2009).

**Statistical Analysis**

Relative gene expression of TLR15 expressed as 40-ΔCt mean values were analyzed by two-way ANOVA considering the tissue and breed/species as effects with Tukey’s post-hoc test using SAS software (version 9.2). Values were considered significant at \( P < 0.01 \).

**Results**

**TLR15 Sequence Analysis**

The nucleotide sequences of TLR15 fragments were aligned to generate full-length ORF sequences, which were submitted to NCBI Genbank. Accession numbers were obtained for Aseel (HM773174), Kadaknath (HM773175) and Japanese quail (HM773176). All three coding sequences had same ORF length of 2,607 bp similar to that of broiler chicken encoding 868 amino acids with molecular weight of 98.2, 98.2 and 98.1 kDa respectively.

By using Clustal (W) analysis, Japanese quail, Aseel and Kadaknath sequences showed 95.1%, 99.7% and 99.8% similarity with broiler chicken TLR15 coding sequence at nucleotide level respectively.

**TLR15 Protein Structure Analysis**

Structure of TLR15 protein of indigenous chicken breeds and Japanese quail were predicted based on deduced amino acid sequences from their respective coding sequences using SMART and TMHMM programmes. In both Japanese quail and indigenous chickens first 22 amino acids constituted signal peptide region, followed by ectodomain region that covered over 652aa residues, transmembrane region (from 654 to position 767) and cytoplasmic TIR domain consisted of 144 residues (from 706 to position 849). SMART prediction revealed that ectodomain of indigenous chickens contained 10 leucine-rich repeats (LRRs) similar to broiler chicken, whereas Japanese quail had 11 LRRs implying minor differences across the species (Fig. 1). There were no N-terminal cysteine clusters in TLR15 in all three sequences studied.

---

**Fig. 1.** Alignment of TLR15 amino acid sequences deduced from full-length ORF sequences of Aseel, Kadaknath, Japanese quail and broiler chicken. LRRs predicted by SMART are shown as gray shadow and boxes indicate predicted glycosylation sites. Bold letters are the predicted signal sequences and arrows indicate domains.
Fig. 1. continued.

<table>
<thead>
<tr>
<th>PPQTVQIAISLVRNQAGTPIESLPESVKHLKVCNCSIVELPEWFANRMOELLFLDLSSN</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aseel</td>
</tr>
<tr>
<td>K</td>
<td>Kadaknath</td>
</tr>
<tr>
<td>Q</td>
<td>Quail</td>
</tr>
<tr>
<td>RISMPDLPISLQLDISNDSIKIPRFKLSTNEVNIQNKLTMEHPFPEYFSTTLTC</td>
<td>Chicken</td>
</tr>
<tr>
<td>I</td>
<td>Aseel</td>
</tr>
<tr>
<td>L</td>
<td>Kadaknath</td>
</tr>
<tr>
<td>Q</td>
<td>Quail</td>
</tr>
<tr>
<td>DISKNLKLVLKALENLESLNVSNLLTIREPACCLPSLTLDSHSLNISLPDHLGC</td>
<td>Chicken</td>
</tr>
<tr>
<td>A</td>
<td>Aseel</td>
</tr>
<tr>
<td>L</td>
<td>Kadaknath</td>
</tr>
<tr>
<td>Q</td>
<td>Quail</td>
</tr>
<tr>
<td>SSLMLKHHNLSCNGKISFLQRSGSLPASLEELDDSDNAITTTIVQDTEGQTLTSVLTVQKH</td>
<td>Chicken</td>
</tr>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
<tr>
<td>FFCNDLWYFVNIYINPHLOINGKDRLCSFPDRRGLVSNSNLHCSLGIQMAIT</td>
<td>Chicken</td>
</tr>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
</tbody>
</table>

**TM domain** ←———————→ **TIR domain**

<table>
<thead>
<tr>
<th>ACMAILVVLVLTLGLCWRFDGGLWVRMWWCMAKRQYYKKRPENKPFDAFISYSEHDADW</th>
<th>Chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
<tr>
<td>TKEHLLKLEDGFKICYHERDFKPGHPVLGNIFYCIESHKVLFLVLSFVNSCWQYE</td>
<td>Chicken</td>
</tr>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
<tr>
<td>LFAEHRVLDNDQDSLIMVLEDLPDSDPQKFSKLRKLRRKTYLKWSPEEHQKIFWH</td>
<td>Chicken</td>
</tr>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
<tr>
<td>QLAAVLKTTEPLVRAENGPNEDVIEME</td>
<td>Chicken</td>
</tr>
<tr>
<td></td>
<td>Aseel</td>
</tr>
<tr>
<td></td>
<td>Kadaknath</td>
</tr>
<tr>
<td></td>
<td>Quail</td>
</tr>
</tbody>
</table>

**TIR domain** ←———————→ **TIR domain**

Fig. 1. continued.
Fig. 2. Phylogenetic relationship of TLR15 with other avian TLR amino acid sequences using Mega version 3.1 following alignment of the sequences using Clustal W and neighbor-joining method (Poisson correction model with pairwise deletion option). Numbers outside the branches indicate the bootstrap values obtained using 1,000 replicates and only values above 50% are shown. The scale bar at the bottom measures the distance. Chicken and Zebra finch TLR are abbreviated as cTLR and fTLR, respectively.

**Phylogenetic Analysis**

Based on the amino acid sequences of TLR15, phylogenetic tree was drawn by MEGA 3.1 considering 1,000 bootstrap values including all chicken TLR sequences and available annotated zebra finch (*Taeniopygia guttata*) TLR sequences (Fig. 2). Phylogenetic analysis by bootstrapping revealed that chicken, Aseel, Kadaknath, Japanese quail and zebra finch TLR15 sequences clustered together and were closer to avian TLR1 family members. **TLR15 mRNA Expression in Different Tissues**

Regression analysis of the standard curve generated by the log10 dilution series gave slope value of 3.39 implying PCR amplification efficiency of 97.2%. There were significant (P<0.01) differences in TLR15 mRNA expression levels between tissues, breed/species and tissue x breed interaction. The expression levels were significantly lower in most of the Japanese quail tissues investigated except for bone marrow than indigenous chicken breeds. Aseel expressed significantly (P<0.01) higher levels of TLR15 than Kadaknath and Japanese quail in most of tissues including bone marrow, bursa, skin and small intestine (Table 1).

**Discussion**

To date 10 TLRs have been found in chicken genome and five of these, TLR2a, 2b, 3, 4, 5 and 7 are directly orthologous to mammals (Temperley et al., 2008). Chicken TLR21 is an ortholog of fish and amphibian TLR21. It appears that TLRs 1LA, 1LB and 15 are unique to birds (Yilmaz et al., 2005; Higgs et al., 2006). The TLR7, 8 and TLR9 subfamily present in fish and mammals is represented only by TLR7 in chicken (Smith et al., 2004; Yilmaz et al., 2005; Temperley et al., 2008). TLR8 is present as pseudogene and TLR9 have been deleted in chicken genome (Philbin et al., 2005). TLR15 is considered as specific to avian, more specifically to chicken as homologues of TLR15 have not yet been found in searches of available vertebrate genome (Higgs et al., 2006). At least TLR15 must exist in avian species if not in other vertebrates. To confirm this speculation, a recent bioinformatic analysis of recently submitted zebra finch (*Taeniopygia guttata*) and turkey (*Meleagris gallopavo*) genome, the currently available avian genome revealed the presence of TLR15 (Cormican et al., 2009; Dalloul et al., 2010). Presence of TLR15 in zebra finch, a passeriform
species, it is evident that TLR15 must have evolved well before the galliformes-passeriformes split (G-P split). Hence, TLR15 must be conserved among the avian lineage.

Chicken TLR15 is molecularly distinct from all known TLRs (Roach et al., 2005; Higgs et al., 2006). The present study confirmed the presence of TLR15 in Japanese quail and indigenous chicken breeds. Analysis of domain structure of TLR15 revealed an archetypal TLR structure comprising of extracellular ligand binding domain including variable number of leucine-rich repeats (LRRs) and the cytoplasmic toll/interleukin-1 (IL-1) receptor (TIR) domain involved in signaling. The length of ectodomain, transmembrane domain and TIR domain were conserved in both the chicken breeds and Japanese quail, although minor differences were found in number of LRRs and their position. The number of LRRs and their phasing vary among TLR family members (Matsushima et al., 2007; Temperley et al., 2008). Indigenous chicken breeds possess 10 LRRs in their ectodomain region similar to chicken sequence, whereas Japanese quail sequence revealed 11 LRRs, one additional LRR at C-terminal and slightly vary in LRRs positions from commercial chicken sequence. In similarity to chicken sequence these LRRs are clustered towards C-terminus of the molecule (Temperley et al., 2008). In contrast to other vertebrate TLRs, there are no N-terminal cysteine clusters in TLR15, which forms a cap like structure to shield the hydrophobic core of first LRR. Interestingly, this feature is shared by vertebrate TLR1 family (TLR1, TLR2, TLR6 and TLR10) too (Matsushima et al., 2007). Furthermore, chicken TLR15 is coded by a single exon, a feature common to all mammalian members of TLR1/2/6/10 clade. Comparing the ectodomain of vertebrate TLR7 family (TLR7, TLR8 and TLR9) to chicken TLR15 revealed some similarity. Chicken TLR15 possess two horseshoe domains of LRRs, a feature observed only in vertebrate TLR7 family. Moreover, LRR of TLR7 family and TLR15 forms deeper arc rather than flat arc as that of other TLRs (Matsushima et al., 2007). However the significance of these common features in ligand recognition cannot be predicted at this stage.

Phylogenetic analysis in the present study which included all chicken and annotated zebra finch sequences revealed TLR15 of Japanese quail and chicken grouped with TLR1 family with high bootstrap support reinforcing the results of earlier studies (Higgs et al., 2006; Temperley et al., 2008). However, it is unlikely that, one to one orthology can ever be drawn convincingly from these identities, along with the pattern in phylogenetic tree. From the phylogenetic tree it can be implicated that chicken TLR1 family may cover the function of mammalian TLR 1/6/10. It can also be speculated that TLR15 may compensate the lack of variability of avian TLR1 family.

The ligand specificity for TLR15 has not yet been conclusively determined, but there are substantial evidences that TLR15 recognizes some components of Salmonella (Higgs et al., 2006; MacKinnon et al., 2009; Nerren et al., 2009). Transcripts of TLR15 increased in tissues of S. enterica serovar Typhimurium infected birds (Higgs et al., 2006). Moreover, TLR15 mRNA expression was significantly higher in caecum of S. enterica serovar enteritidis infected birds (MacKinnon et al., 2009). The basal levels of mRNA expression of TLR15 were greater in heterophils from Salmonella- resistant chickens than in heterophils from Salmonella- susceptible chickens (Nerren et al., 2009). There is also some speculation that TLR15 may form heterodimer with TLR2 as gene expression pattern of TLR2 and TLR15 mRNA was highly similar in Salmonella-infected chicken (Higgs et al., 2006; Mackinnon et al., 2009). Dimerization of vertebrate TLR1 family members with TLR2 during the course of ligand recognition is well established (Keestra et al., 2007; Higuichi et al., 2008; Uematsu and Akira, 2008). Collectively these studies strongly suggest that TLR15 plays a role in immune response to multiple serovars of Salmonellae. In a recent study in vitro stimulation of chicken heterophils with purified TLR agonist, heat killed and formalin inactivated whole bacteria demonstrated that individual TLR agonists are not the ligand for TLR15. The authors speculated that TLR15 recognize unique, non-secreted, heat-stable component of both gram (+)

### Table 1. Relative transcript expression level of TLR15 in Aseel, Kadaknath and Japanese quail in a range of tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Breed/Species</th>
<th>Aseel</th>
<th>Kadaknath</th>
<th>Japanese quail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td>37.05±0.05f</td>
<td>39.41±0.09b</td>
<td>30.16±0.03b</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td>38.45±0.13c&lt;sup&gt;ds&lt;/sup&gt;</td>
<td>39.92±0.03c&lt;sup&gt;s&lt;/sup&gt;</td>
<td>37.15±0.02f&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small Intestine (SI)</td>
<td></td>
<td>39.03±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>37.74±0.31&lt;sup&gt;f&lt;/sup&gt;</td>
<td>31.80±0.22&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td>39.44±0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.27±0.75&lt;sup&gt;ds&lt;/sup&gt;</td>
<td>37.35±0.21f&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bone marrow (BM)</td>
<td></td>
<td>39.44±0.18&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>37.26±0.07&lt;sup&gt;f&lt;/sup&gt;</td>
<td>38.39±0.19&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bursa</td>
<td></td>
<td>39.46±0.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38.55±0.06&lt;sup&gt;ed&lt;/sup&gt;</td>
<td>38.17±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data shown are mean ±SE. Values with different superscripts differ significantly (P<0.01). SI- small intestine, BM-bone marrow.
and gram (−) bacteria of avian specific pathogens (Nerren et al., 2010).

Although some data are available regarding the expression status of TLR15 in different tissues of commercial broiler chicken (Higgs et al., 2006; Meade et al., 2009), the status of expression in Japanese quail and indigenous chickens are not known. In the present study, TLR15 is expressed in multiple tissues including both lymphoid and non-lymphoid tissues throughout the body rather than being limited to particular site similar to earlier report (Higgs et al., 2006). In general, the relative level of expression of TLR15 transcripts was significantly lower in Aseel chicken breeds, and expressed in multiple tissues, including both lymphoid and non-lymphoid tissues throughout the body rather than being limited to particular site similar to earlier report (Higgs et al., 2006). Moreover, implication of TLR15 expression in various tissues could be convincingly drawn only after confirmation of its ligand specificity. Moreover implication of TLR15 expression in various tissues could be convincingly drawn only after confirmation of its ligand specificity. In conclusion, the present investigation characterized TLR15 coding sequence of Japanese quail and indigenous chicken breeds and profiled the expression pattern of its transcripts in various tissues.

References


Mohapatra SC. Poultry production. In Handbook of Animal
Nerren JR, He H, Genovese K and Kogut MH. Expression of
the avian-specific toll-like receptor 15 in chicken heterophils
is mediated by Gram-negative and Gram-positive bacteria,
but not TLR agonists. Veterinary Immunology and Immu-
Nerren JR, Swaggerty CL, MacKinnon KM, Genovese KJ, He
H, Pevzner I and Kogut MH. Differential mRNA expres-
sion of the avian-specific toll-like receptor 15 between
heterophils from Salmonella-susceptible and resistant chick-
Panda B and Mohapatra SC. Poultry production. ICAR, New
Philbin VJ, Iqbal M, Boyd Y, Goodchild MJ, Beal RK,
Bumstead N, Young J and Smith AL. Identification and
characterization of a functional, alternatively spliced Toll-
like receptor 7 (TLR7) and genomic disruption of TLR8 in
Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith
KD, Hood LE and Aderem A. The evolution of vertebrate
Toll-like receptors. Proceedings of the National Academy of
Sciences of the United States of America, 102: 9577–9582.
2005.
Rout PK, Pani PK and Naithani S. Genetic susceptibility of
indigenous chicks to subgroup A Rous sarcoma virus in-
oculated via the chorioallantoic membrane. Veterinary Im-
Schultz J, Milpetz F, Bork P and Ponting CP. SMART, a simple
modular architecture research tool: identification of signal-
ing domains. Proceedings of National Academy of Sciences
Smith J, Speed D, Law AS, Glass EJ and Burt DW. In-silico
identification of chicken immune-related genes. Immuno-
genetics, 56: 122–133. 2004.
Takeda K and Akira S. Toll-like receptors in innate immunity.
Temperley ND, Berlin S, Paton IR, Griffin DK and Burt DW.
Evolution of the chicken Toll-like receptor gene family: a
2008.
Uematsu S and Akira S. Toll-Like receptors (TLRs) and their
2008.
West AP, Koblansky AA and Ghosh S. Recognition and signal-
ing by toll-like receptors. Annual Review of Cell and Devel-
Wimmers K, Ponsuksili S, Hardge T, Valle-Zarate A, Mathur
PK and Horst P. Genetic distinctness of African, Asian and
South American local chickens. Animal Genetics, 31: 159–
165. 2000.
Yilmaz A, Shen S, Adelson DL, Xavier S and Zhu JJ. Identifica-
tion and sequence analysis of chicken Toll-like receptors.