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Expression profiles of Toll-like receptors 1, 2 and 5 in selected organs of commercial and indigenous chickens

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Abstract Toll-like receptors (TLRs) are members of the cellular receptors that constitute a major component of the evolutionary conserved pattern recognition system (PRR). TLRs are expressed in a wide variety of tissues and cell types. In this study we compared the expression profiles of the chicken TLR1, TLR2 and TLR5 genes in a range of organs (lung, ovary, liver, thymus, duodenum, spleen and large intestine) in commercial Hy-Line (HL) and indigenous Green-legged Partridgelike (GP) chickens. The level of mRNA was determined with RT-qPCR using the TaqMan probes for target and reference (ACTB) genes. We determined that the tissue profiles differed with respect to each TLR and they were ranked as follows: spleen, lungs, large intestine (TLR1), large intestine, lungs, thymus/ovary (TLR2) and lungs, thymus, liver (TLR5). A differential expression between HL and GP chickens was determined for TLR1 and TLR5 genes in large intestine and thymus of HL ($P<0.05$) and GP ($P<0.05$) chickens. We conclude that the commercial chickens expressed higher levels of TLR1 mRNA in large intestine and TLR5 mRNA in thymus than indigenous chickens.

Keywords Chicken · Innate immunity · RT-qPCR · Tissue profiling · TLR

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Toll-like receptors (TLRs) are members of cellular receptors that constitute a major component of the evolutionary conserved pattern recognition system (PRR). They recognize microbial infectious antigens referred to as pathogen-associated molecular patterns (PAMPs). TLRs are expressed in a wide variety of tissues and cell types, such as macrophages, monocytes, dendritic cells, B and T cells, mast cells, NTK, neutrophils, eosinophils, fibroblasts, intestinal epithelium, endothelium of veins, smooth muscle cells, etc. (Yilmaz et al. 2005; Iqbal et al. 2005; Becker and O'Neill 2007; Kannaki et al. 2010). In particular, TLRs are present in the organs which participate in the immune responses and in the tissues that are commonly exposed to pathogens, including skin, respiratory tract, intestinal and genitourinary tracts, bladder, kidney, spleen and thymus (reviewed by Becker and O'Neill 2007). Expression of the TLRs in chickens is strongly affected by the physiological status of the organisms (Abasht et al. 2009; Rodríguez-Lecompte et al. 2012, reviewed by St. Paul et al. 2013). Besides, it has been reported that genetically distant chicken lines (broiler, layer, indigenous Indian chicken) show different patterns of the TLRs genes expression (Abasht et al. 2009; Ramasamy et al. 2010). The aim of this study was to compare the expression profiles of the chicken TLR1, TLR2 and TLR5 genes in a range of organs in commercial (Hy-Line) and indigenous (Green-legged Partridgelike) chickens.

The experiment was performed on 4-weeks old Hy-Line (HL, commercial layer) and Green-legged Partridgelike (GP, indigenous) chickens. Three random female chickens per breed accounted for individual biological replicates. Samples of the lung, ovary, liver, thymus, duodenum, spleen and large intestine were used for RNA extraction using TRI reagent (Invitrogen, Carlsbad, CA, USA). RT-qPCR method was used for determination of the mRNA levels of TLR1 type 1 (chTLR1La, denoted as TLR1),

TLR2 type 2 (chTLR2b, denoted as TLR2) and TLR5 genes. cDNA was synthesised with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). PCR primers and TaqMan probes were designed in Primer Express 3.0 programme (Applied Biosystems, Foster City, CA, USA). β-actin (ACTB) was used as the reference gene. Table 1 includes the details of the qPCR assays. RT-qPCR reactions were performed in triplicates, in 20 μl reaction volume, using TaqMan Universal PCR Master mix with UNG (Applied Biosystems, Foster City, CA, USA), 200 nM of each primer and 100 nM of TaqMan probe (Applied Biosystems, Foster City, CA, USA). The thermal cycling was done on ABI PRISM TM 7700 (Applied Biosystems, Foster City, CA, USA). The comparative $^{\Delta\Delta}$Ct (Livak and Schmittgen 2001) method was used to determine fold-changes in gene expression. Analysis of variance was performed on $^{\Delta}$Ct values ($^{\Delta}$Ct = Ct target gene–Ct ref gene) with one-way ANOVA followed by post-hoc Duncan's test (tissue profiling), and with a Student's *T*-test (comparison between chicken breeds).

The results of the expression analysis of the three TLR genes are presented in Fig. 1. Chicken organs characterized by the high levels of relative expression of the TLRs were ranked as follows: spleen, lungs, large intestine (TLR1), large intestine, lungs, thymus/ovary (TLR2) and lungs, thymus, liver (TLR5). Those observations are in agreement with the available literature. Tissue profiling of TLRs genes' expression in chicken (Iqbal et al. 2005; Yilmaz et al. 2005; Dhinakar Raj et al. 2009; review by Kannaki et al. 2010) confirm that spleen, lungs, thymus and liver express high levels of the respective TLRs.

Regarding the two chicken breeds analyzed, the highest level of TLR1 in HL chickens was determined in spleen, lungs and intestines ($P<0.05$). However, in GP chicken this gene was highly expressed only in spleen and lungs ($P<0.05$) (Fig. 1a). Chicken TLR1 type 1 and 2 is involved in recognition of peptidoglycan and lipoprotein of mycobacteria (Higuchi et al. 2008). The profiles of TLR2 were comparable between breeds (Fig. 1b). Large intestine and lungs had the highest level of TLR2 mRNA in both HL and GP chickens ($P<0.05$). Chicken TLR2 had been shown to bind a wide range of ligands present on a variety of microorganisms, including Gram-positive and Gram-negative bacteria, fungi, viruses and parasites (St. Paul et al. 2013) and the literature reported expression of TLR2 type 2 in all tissue types (Fukui et al. 2001; Iqbal et al. 2005; Yilmaz et al. 2005), which is consistent with our study. Furthermore, the tissue profiles of TLR5 expression differed between HL and GP chickens (Fig. 1c). TLR5 plays an important role in the host defence against bacterial pathogens, by recognition of flagellin, which is a subunit of the bacterial flagellum. It is considered a crucial component involved in fighting various bacterial infections, such as flagellated *Salmonella enterica* (Vijay-Kumar et al. 2008).

In our study, a differential expression of TLR1 and TLR5 was determined in large intestine and thymus of HL and GP chickens. Both those genes had been proven to undergo positive Darwinian selection, especially with respect to leucine-rich repeat (LRR) domain, which is responsible for molecular recognition (Ruan et al. 2012; Yilmaz et al. 2005). Interestingly, in our study the significant differences in the TLRs profiles (higher expression of TLR1 in large intestine and TLR5 in thymus), are mostly in favor of HL breed–commercial layer selected for productivity traits–than indigenous GP. The basal expression pattern of TLRs' mRNAs indicates

Table 1 Molecular assays used for relative quantification of the chicken TLR1, TLR2 and TLR5 genes with RT-qPCR

Gene	ID	Molecular assays sequences (5'→3')
TLR1[a]	426274	F: GTCATCTGTTGGCTCTTCACTCA
		R: CATATATAGGTTTTCTGTTCAGTATGCCTCA
		P: CCATAAAAGGTTGGACTTCT
TLR2[b]	374141	F: ACTGCCTGCAACGGTCAT
		R: CATCAGCTTCATTGTTGGTTTCTGT
		P: CTCAGCTACACCAAAATG
TLR5	554217	F: ACACGGCAATAGTAGCAACACATAT
		R: ACACCTGGAACTTGGAAAAGAACAT
		P: TTTGCGAGCCAGTTTC
ACTB	396526	F: CCCCGAGGCCCTCTTC
		R: GATGGAGTTGAAGGTAGTTTCATGGA
		P: CTTTCTTGGGTATGGAGT

[a] chTLR1 La

[b] chTLR2b (nomenclature by Temperley et al. 2008)

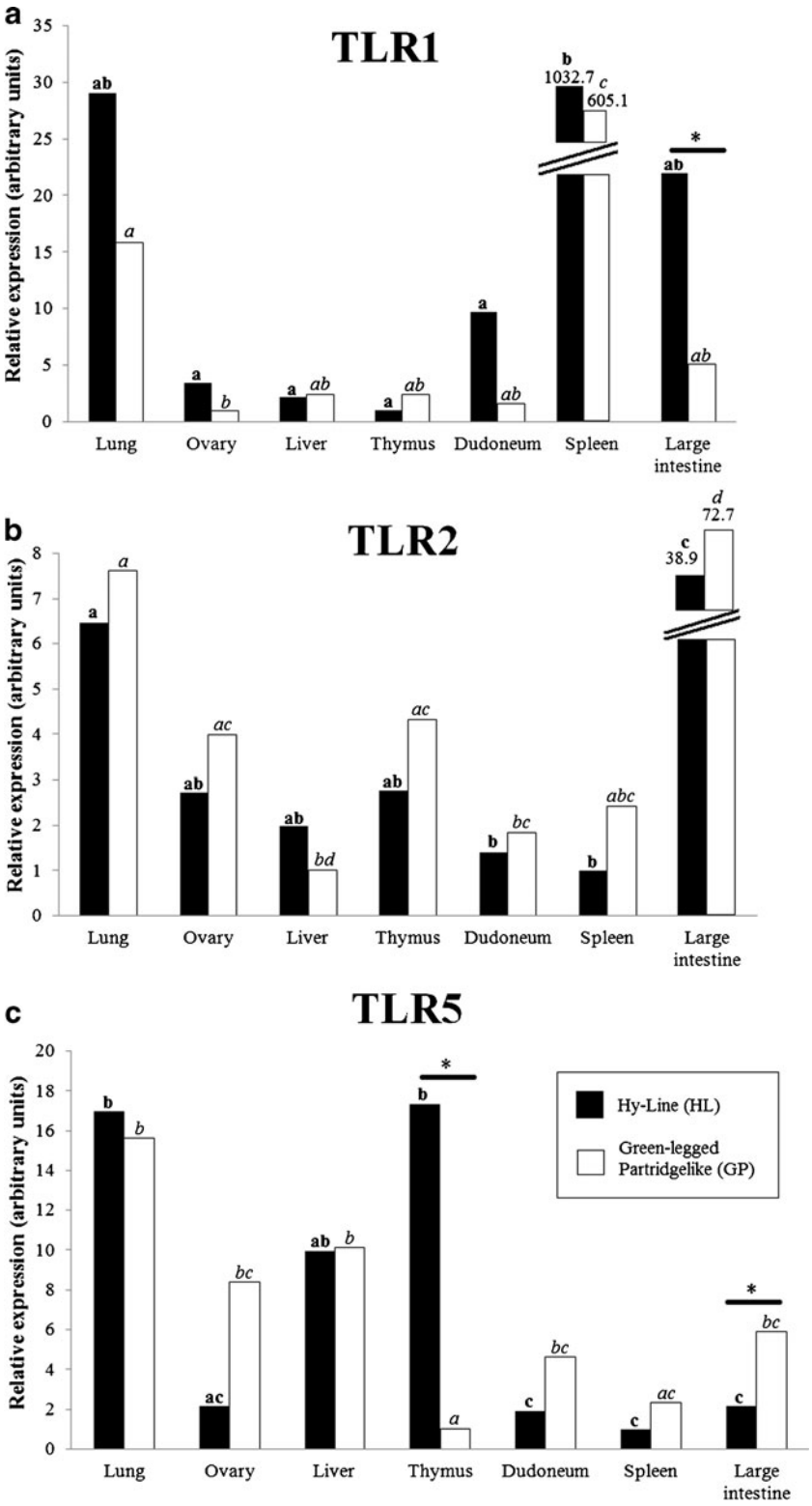

Fig. 1 Tissue profiling in Toll-like receptors (TLR)1, TLR2, and TLR5 mRNA expression level in selected organs (lung, ovary, liver, thymus, dudoneum, spleen and large intestine) of commercial layer (Hy-Line) and indigenous chicken (Green-legged Partridgelike). Bars represent fold-changes in the gene expression in different organs of HL (*black bars*) and GP (*white bars*) chickens, calculated by $^{\Delta\Delta}$Ct method. Bars, within the breed, that do not share a common letter are significantly different ($P<0.05$, Duncan's test). Pairwise differences between HL and GP chickens were marked with the *asterisk* ($P<0.05$, T-Student's test)

the natural PAMPs' load of each tissue and its ability to resist pathogen challenge. Since all experimental animals were raised in the same environment, we conclude that the differential expression of TLR1 and TLR5 indicate that HL and GP chickens developed a different genetic adaptation to respond to pathogen burden through PRR.

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