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Expression analysis of viral nucleic acid recognizing toll-like receptor-7 and 8 genes across caprine tissues

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Toll-like receptors (TLRs) play a crucial role in innate as well as adaptive immunity. Roach *et al*. (2005) reported that 13 such structurally related mammalian receptor proteins have been identified. Among these reported TLR's, TLR7 and TLR8 recognize viral single-stranded RNA ligands. Variations in expression of TLRs was associated with immune responsiveness and disease progression (Helmby and Grencis 2003). So to understand how the tissues and cells generate the response in detection of pathogens, TLRs expression pattern needs to be determined. TLR expression was analysed by using semi-quantitative PCR in chickens (Iqbal *et al*. 2005), bovines and ovines (Menzies and Ingham 2006), buffalo (Vahanan *et al*. 2008) and goat (Thirumurugan *et al*. 2009). Goat plays significant role in agrarian economy of India, but when it comes to genomics part not much work has been carried out on caprines and this is the first report on documentation of distribution of caprine TLR7 and TLR8 genes using real-time PCR assay across different tissue samples.

Eleven different tissues (liver, lungs, kidney, skin, ovary, spleen, lymph-node, heart, mammary-gland, intestine and skeletal muscle) of a healthy goat were collected from Delhi abattoir (Ghazipur Slaughter House) and were kept in RNALater/liquid nitrogen, till brought to lab. Total RNA was isolated from approximately 250 mg of homogenised tissue by TRIzol method following the manufacturer's instructions. The extracted RNA was further purified and treated with DNaseI using RNeasy kit and was quantified by UV spectrophotometer considering the OD_{260}/OD_{280} ratio, which was 1.9–2.0 for all the samples. Reverse transcription was performed using oligo(dT) primers from 2 µg of the total RNA of each sample using the, First Strand cDNA synthesis kit, using the protocol given by the manufacturer. TLR7 and TLR8 gene specific primers, designed from the already available mRNA sequence of buffalo were used. Primers for house-keeping gene, RPS15 were designed from mRNA sequence of *Bos taurus* (Table 1). Real-time PCR was performed using 96-well LightCycler480 real-time PCR System using qPCR master mix according to manufacturer's instructions. Standard curve for the housekeeping gene RPS15 was drawn on 10-fold dilutions of cDNA. Dissociation curve analysis performed for each assay to ascertain the specific amplification. No-template controls

Table 1. Primer sequence and other details of real-time PCR performed for expression analysis of TLR7, TLR 8 and internal control RPS 15 genes of goat

Gene	Accession number	Primer sequence	Annealing temp. (°C)	Size (bp)
TLR7	GU903503.1	F: 5'TTGAGGAAAGGGACTGGTTACC 3' R: 5'GTCTTTGCGTACTTGTCTGTCATCA 3'	57	108
TLR8	GQ499855.1	F: 5'ACCTGCCGTCTGATTTTCTTTCTG 3' F: 5'GGTGGCGGTCTTCGTTTCAA 3'	56	147
RPS15	NM_001192201.1	F: 5'CAGCTTATGAGCAAGGTCGT 3' R: 5'GCTCATCAGCAGATAGCGCTT3'	56	130

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were also run for each primer. Two technical replicates for each sample were used for real-time PCR. Gene expression values were calculated for each reference gene using delta-Ct method, mean threshold Ct values of at least two replicates for each tissue were determined and the mean results were expressed in the form of mean normalized expression, subtracting the values of housekeeping gene.

TLRs expression determines the individual's ability to respond against the pathogens and also gives the ability to differentiate self from non-self. TLRs also have the ability to specifically recognize the invading pathogens from the commensals. There are many factors such as location and the abundance of each TLR potentially involved which determine their role in immunity as well as inflammatory diseases (Cario *et al*. 2002). Due to this reason TLR7 and TLR8 genes expression profiling was carried out in various tissues of Indian goats. These 2 TLRs were selected since both help in recognizing RNA virus ligands and in Indian goats severe outbreaks of *Peste des petits ruminants* (PPR), a disease caused by ssRNA virus causing heavy economic losses to goat farmers is reported (Malik *et al*. 2011).

Among the 2 TLRs studied, TLR7 was found to be expressed in all the 11 goat tissues taken for the present study (Table 2). TLR7 showed maximum expression in liver followed by kidney, spleen, lungs, ovary, lymph node and mammary gland whereas relatively lower expression was found in heart, skin, intestine and skeletal muscle. In bovines however, skin has shown highest expression of TLR7 (Menzies and Ingham 2006). Buffalo TLR7 expression was seen in all the cells and tissues analyzed by other workers (Vahanan *et al*. 2008). Also in the earlier reports by semi-quantitative RT-PCR it has been reported that tissues such as the lungs, skin, uterus and jejunum express consistently higher levels of TLR7 in goat (Thirumurugan *et al*. 2010). In mice the TLR7 was found to be expressed differentially throughout the autoimmune disease progression (Patole *et al*. 2006). The expression of TLR7 was found higher in the developing brain of mice out of other ten TLRs studied and it was concluded that these receptors holds the significant role in developmental processes in the Central nervous system (Kaul *et al.* 2012). Tissues such as kidney, lungs were found to have the increased TLR7 mRNA expression whereas, tissues like heart and liver affected with the autoimmune disease, were found to have the reduced level of TLR7 mRNA expression. Since most of viral infections like PPR in goats have respiratory mucosal route of entry, lungs and immune organs like spleen, lymph nodes are expected to be having relatively higher expression of TLR7 as found in this study.

Goat TLR8 was also found to be expressed in all the tissues studied (Table 2). The highest repertoire of TLR8 was found to be expressed in kidney followed by spleen, lymph node, lungs, ovary, liver and mammary gland, whereas, lower expression was seen in intestine, skeletal muscle, heart and skin. Goat skin previously has been reported to have the lowered expression of TLRs 2, 4, 8 mRNAs (Thirumurugan *et al*. 2010) and the results observed in lungs were consistent with the findings in human and mouse (Brown and Gordon 2001). In buffalo also the TLR8 was found to be expressed in all cells and tissues analyzed except kidney, which showed absence or lower expression (Vahanan *et al*. 2008). In swine also heart was found to be showing expression of invariably all types of TLRs, however lower as compared to the kidney, spleen, lymph node, liver and lungs for TLR8, the results were in continuity with our results where kidney showed the maximum expression and the heart showed the lower (Veeresh *et al.* 2012). The increased expression of TLR7 and TLR8 in lymphoid tissues in PRRSV-infected pigs has been reported by Liu *et al*. (2009), confirming their important role in clearance of ssRNA viruses. So the cell type or tissue type-specific response will depend on the distinct TLR expression profile of such cells or tissues, since the regulation of TLRs can be related either to specific promoter-binding elements or to cell-specific expression of additional regulatory factors (Liew *et al*. 2005).

Interestingly, the contact between a virus/bacteria and a TLR-expressing cell is often sufficient to induce type I IFN production without need for infection, allowing uninfected cells to participate in the antiviral responses (Malmgaard *et al*. 2004). Although the importance of an organ or tissue cannot be assumed by the presence of TLRs only, as many tissues consist of different cell types and a minimum number of specific cell types which may not be important in immune recognition, but their pattern recognition receptors may not be detected by the whole organ analysis. Their distribution in different mammalian species has revealed characteristic patterns of expression for each TLR. The tissue and cell distribution of TLRs is thus important to assess their functions since it influences the capacity of individual to detect different micro-organisms during their entry as well as growth in different tissues.

Tables 2. Normalized Delta Ct values of TLR7 and TLR8 genes across different goat tissues (higher the value, lower the expression)

Tissues	DC_t TLR7	DC_t TLR8
Liver	–5.47	–0.645
Lungs	–1.59	–2.56
Kidney	–3.155	–3.575
Skin	3.155	0.855
Ovary	–1.42	–0.72
Spleen	–2.635	–3.125
Lymph-node	–1.25	–2.63
Heart	7.885	0.585
Mammary-gland	–0.965	–0.005
Intestine	1.58	4.57
Skeletal muscle	1.255	1.185

This study thus shows variable distribution of both TLR7, TLR8 genes in different goat tissues using highly sensitive real-time PCR technique for the first time. The results could be useful in further studying the role of these two TLRs in mechanism of innate immune response generation against viral pathogens in goat.

SUMMARY

The present study was undertaken to analyze the expression of TLR7 and TLR8 genes of goat in different tissues by real-time PCR technique. Although the expression of both the genes was observed in all the 11 different tissues taken, maximum expression of TLR7 was observed in liver, followed by kidney, lungs, lymph node and lowest expression was found in heart. TLR8 showed maximum expression in kidney followed by spleen, lymph node, lungs and the lowest expression in intestine and skin. The findings suggested that both the genes are having important functions in kidney and lungs apart from immune organs in goat.

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