India is one of the major bio-diversity centers of the world in domestic livestock and poultry genetic resources. There are over 37 registered breeds of cattle, 13 breeds of buffalo, 39 breeds of sheep, 23 of goat, 8 of camel, 6 breeds of horse and 15 of indigenous poultry (http://www.nbagr.res.in/regchi.html), besides majority of distinct populations not classified as a descript breeds. These livestock species play important role not only to meet the nutritional requirements of the country but are also considered important from economic point of view (Ranjhan and Pathak, 1978). Among these, goat is one of the important livestock species, having major contribution in meat, skin and milk production and was earliest ruminant species to be domesticated (Hatziminoglou and Boyazoglu, 2004). As indicated by the archaeological evidence, goats have been associated with man in a symbiotic relationship for more than 10,000 years back (Ensminger and Parker, 1986).

With more than 140 million population and 23 descript, registered breeds, goats account for more than 25 per cent of the total livestock in our country, having the second-largest goat population in the world after China. The domestic goat (Capra hircus) is an important species in India and other developing countries because of the low investment, wide adaptability, high fertility and fecundity, low feed and management needs, high feed conversion efficiency, quick pay-off and low risk involved (Manjunath et al., 2004). It plays an important role in income generation, capital storage, employment generation and improving household nutrition and also provides food and nutritional security to the millions of marginal and small farmers and agricultural labourers (Singh and Kumar, 2007). The importance of this valuable genetic resource is underestimated as they are often neglected in comparison to cattle and sheep.

The diverse populations of Indian goats are well adapted to local climatic conditions and can survive on scarcely available forage and fodder. Of the total meat production, more than 70 percent comes from cattle, buffalo and pig and for those species preference is limited due to socio-religious factors. Therefore, burden lies on goat and sheep meat (Birthal and Joshi, 2006). But the productivity of Indian goat is assumed to be low and major reasons for this low productivity are: inadequate grazing resources, disease problems and serious lack of organized efforts for genetic improvement. As disease being the major reason for low productivity, it is necessary to target genes involved in disease
resistance to understand immune response mechanism and to develop proper preventive measures.

In mammals, there are two fundamentally different types of responses of the immune system; innate and acquired or adaptive. Primarily, innate defence mechanism reduces pathogen load and provides time for the development of highly specific and long-lasting adaptive immune response, characterized by high diversity, specificity and memory. Some cells of the innate immune system, such as macrophages and dendritic cells bear non-clonally distributed receptors termed as pattern recognition receptors (PRRs). These PRRs recognize microbial conserved domains called as pathogen associated molecular patterns (PAMPs), which are essential for the survival of the micro-organisms and are therefore difficult to alter (Griebel et al., 2005). Among these, toll-like receptors (TLRs) constitute a multi-gene family of PRRs in vertebrates genome and are classified on the basis of recognition of distinctive PAMPs, playing a key role in protection against both viral and bacterial infections (Carpenter and O’Neill, 2007; Krishnan et al., 2007).

Toll protein was first discovered in drosophila in 1985, governing dorsal ventral polarity and immune response against fungal infection (Anderson et al., 1985). Toll-like receptors recognize different types of pathogens and play significant role in innate as well as adaptive immunity and are mainly expressed on a number of immune cells such as macrophages, dendritic cells and B-lymphocytes (Faure et al., 2001). PAMPs (lipoproteins, lipopolysaccharides, flagellin, dsRNA, etc.) are the conserved features of pathogens, recognized by different types of TLRs (Kaisho et al., 2006). Structurally TLRs consist of two domains, extra-cellular domain having leucine rich repeats (LRRs), involved in PAMP recognition and intracellular Toll/interleukin-1 receptor domain (TIR domain) involved in signal transduction (Matsushima et al., 2007; Watters et al., 2007). Distribution of ligand recognition region of TLRs at cellular level follows certain unique features with that of TLRs 1, 2, 4, 5 and 6 being present on cell membrane, while that of TLRs 3, 7, 8 and 9 being found within the cytoplasm.

Although over the past few years, considerable progress has been made to understand the role of TLRs and their association with disease resistance and susceptibility in many species, but still relatively little is known about TLRs contribution in host defense among livestock species. TLR genes have now been identified in a number of vertebrate genomes, and many partial and full length sequences are available. Till date 13 TLRs have been discovered in mammals, out of which 10 have been well identified and characterized in human, mouse, cattle, sheep, chicken and pig (Chang et al., 2009). The two major
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classes in which TLRs are mainly divided: one are called as bacterial TLRs which includes TLR1, 2, 4, 5, 6 and other class is called as viral TLRs which includes TLR 3, 7, 8 and 9, since the major pathogens which cause diseases among the livestock species are viruses and bacteria. So it becomes very important to study the role of such immune response genes, which respond against these pathogens.

Several polymorphic nucleotides (single nucleotide polymorphism, SNPs) have been detected in various TLR genes of different species, which have been associated with immunity against many microbial infections (Nicolas et al., 2005; Shinkai et al., 2006; Leyva-Baca et al., 2007), but still little is known about the goat TLR genes. More recently, full-length sequencing and characterization of all the 10 TLR genes have been reported in goat (Raja et al., 2011). Among these, TLR4 is the one which recognizes lipopolysaccharides, a cell-wall component of Gram-negative bacteria and TLR8, which recognizes viral nucleic acids as ligand and both of them are very important in mounting innate as well as systemic immune response against two types of pathogens. Polymorphism in TLR4 has been associated to various bacterial diseases in various species such as mastitis and paratuberculosis in bovine (Pinedo et al., 2009), HIV in humans (Papadopoulos et al., 2010) etc. and polymorphism in TLR8 has also been associated with higher susceptibility to asthma, TB and HIV infections in human (Davila et al., 2008).

Though the knowledge of the innate immune mechanism and signaling mediated through TLRs could provide more insights into the disease resistance or susceptibility, but still the work in this field is in its infancy in the livestock species like goat. Therefore keeping in mind the importance of goat species to our agrarian economy and role of TLR genes in disease resistance, it is relevant to characterize the TLR genes of goat through sequence analysis, SNP detection and their expression. Hence, the present work was proposed with the objectives:

1) To characterize Toll-like receptor genes (TLR4 and TLR8) of Indian goats by sequencing and further comparison with other livestock species.

2) To detect polymorphism in the TLR4 and TLR8 genes of Indian goats and to analyse their tissue distribution by expression analysis using Real-Time PCR.

3) To clone Toll-like receptor 4 and 8 genes of goat for expression in prokaryotic expression vectors and to characterize the expressed proteins.