



## Expression Profile of CXCL3 Gene in Peripheral Blood Mononuclear Cells Challenged *in vitro* with *Theileria annulata* in Crossbred Cattle

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### ABSTRACT

Bovine tropical theileriosis is a major haemoprotozoan disease associated with high rates of morbidity and mortality particularly in exotic and crossbred cattle. Recent studies suggest that a number of immune response genes, expressed differentially in exotic and indigenous breeds play an important role in breed specific resistance to tropical theileriosis. In the present study, expression of CXCL3 gene which has chemotactic activity for neutrophils, controls migration and adhesion of monocytes and ultimately mediates its effects on target cells by interacting with a cell surface chemokine receptor called CXCR2 was studied in crossbred cattle. The *in vitro* experimental result revealed significant difference in CXCL3 gene expression in *Theileria annulata* challenged peripheral blood mononuclear cells of crossbred animals as compared to healthy controls and a 2.53 fold increase ( $p < 0.05$ ) was recorded. The results of current study indicate that CXCL3 may be involved in host-pathogen interaction during tropical theileriosis.

**Keywords:** Crossbred, *Theileria annulata*, CXCL3, PBMCs

Bovine tropical theileriosis is a debilitating disease caused by the protozoan parasite *Theileria annulata*, transmitted by *Hyalomma anatolicum anatolicum* and has a wide geographical distribution from the Mediterranean basin to China. This disease is associated with high levels of morbidity and mortality, principally in exotic and crossbred cattle as compared to indigenous cattle. The disease identified as a major constraint on the improvement of cattle farming in several developing countries (Minjauw and McLeod, 2003). As per the last estimate, the cost of tropical theileriosis in India is approximately US\$ 384.3 million (Minjauw and McLeod, 2003). The ideal approach to control tropical theileriosis, as with other tick-borne diseases, includes a portfolio of integrated strategies that are economically and environmentally sustainable. An attractive control strategy is to exploit pre-existing genetic resistance (Glass and Jensen, 2007). A study comprised of expression profile of many immune related genes by *in vitro* challenge of peripheral blood mononuclear

cells (PBMCs) with *T. annulata* showed significant differential regulation of genes in crossbreds as compared to indigenous cattle (Dewangan *et al.*, 2015). With the burgeoning information in genetics and genomics, new opportunities to identify factors controlling disease resistance can now be explored (Soller and Andersson, 1998). Chemokines are chemotactic cytokines controlling the migratory patterns and positioning of immune cells. Chemokine (C-X-C motif) ligand 3 (CXCL3) is a small cytokine belonging to the CXC chemokine family, controls migration and adhesion of monocytes and mediates its effects on target cells by interacting with a cell surface chemokine receptor called CXCR2 (Smith *et al.*, 2005; Ahuja and Murphy, 1996). The *T. annulata* parasite infects mainly cells of the myeloid lineage which are also the main producers of inflammatory cytokines. Genes encoding these inflammatory cytokines in macrophages are up-regulated in response to *Theileria* infection (Brown *et al.*, 1995). The present study was conducted with the

**Table 1.** List of Primers used in present study.

Genes	Primers	Primer sequence	Primer length	Amplicon size
CXCL3	RT- CXCL3 F	GCTTGTCTCAACCCTGAAGC	20 bp	139 bp
	RT- CXCL3 R	TCCTCTATGAGCAGAGACCACT	22 bp	
GAPDH	RT-GAPDH F	GGCGTGAACCACGAGAAGTATAA	23 bp	194 bp
	RT-GAPDH R	CCCTCCACGATGCCAAAGT	19 bp	

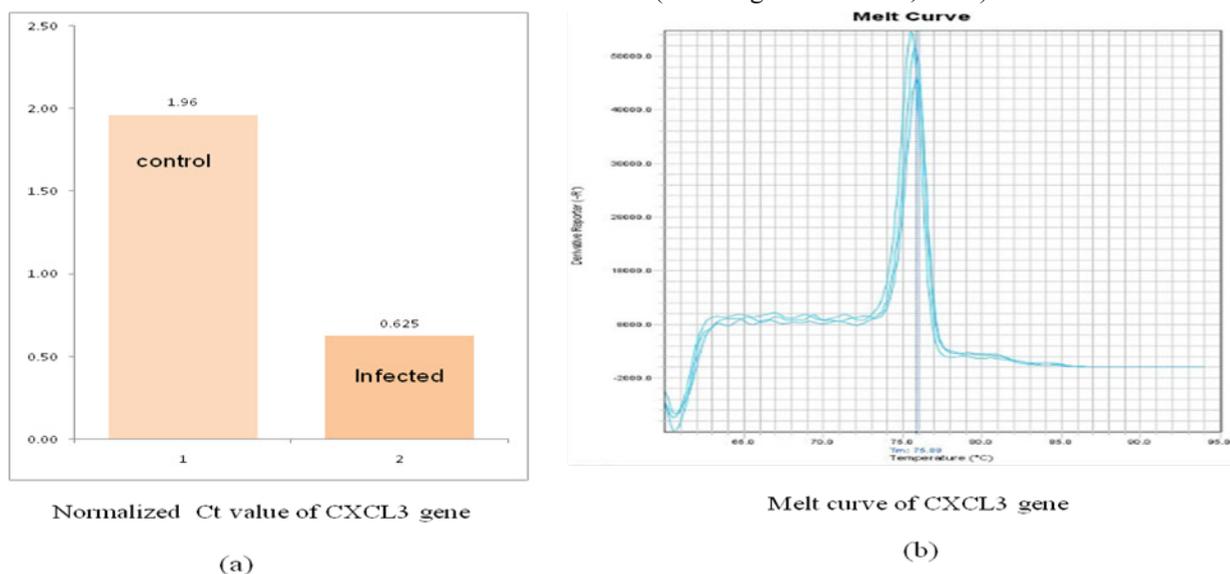
objective to see the changes, if any, in mRNA expression of CXCL3 in PBMCs challenged with *T. annulata* sporozoite in early hours.

Four crossbred calves of around three months of age having good general health maintained at Cattle and Buffalo Farm, Indian Veterinary Research Institute Izatnagar were used in present study. Calves were screened for tropical theileriosis by blood smears examination before proceeding for further experiment.

Peripheral blood was collected aseptically and immediately stored on ice. Peripheral blood mononuclear cells (PBMC) were separated under cold conditions by density gradient centrifugation (Histopaque-1.083 g ml<sup>-1</sup>, Sigma, Poole, Dorset, UK). The cells were resuspended at 2x10<sup>6</sup> cells/ml in RPMI-1640 medium supplemented with 20% FBS and aliquot into a 6-well plate. The *T. annulata* sporozoites, (Parbhani isolate) maintained in the

Protozoology Laboratory, Division of Parasitology, of the institute was used to infect the cells *in vitro*. Sporozoite suspension was mixed at the rate of 0.5 tick equivalents in 2x10<sup>6</sup> cells/ml. The PBMCs were incubated at 37° C in a 5% CO<sub>2</sub> incubator for 2 h.

RNA isolation was done using RNeasy Plus Mini Kit (Qiagen) as per the manufacturer’s instructions. RNA was quantified by NanoDrop ND 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Primers were designed (Table 1) and Real time PCR reactions were performed using Fast SYBR® Green Master mix (Applied Biosystems, Warrington, UK). The qPCR thermal cycling program consisted of one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. A dissociation step was included to confirm amplification specificity. Four biologicals (crossbred calves) and three technical replicates were used in the present study. Data generated were analysed by comparative C<sub>T</sub> method (Schmittgen and Livak, 2008).



**Figure 1.** Normalized Ct value and melt curve of CXCL3 gene.

Significant difference was observed in CXCL3 gene expression in crossbred animals. A 2.53 fold change ( $p < 0.05$ ) was found in *T. annulata* infected PBMCs as compared to control PBMCs (Fig. 1a). Melt curve showed the desired amplification specificity (Fig. 1b).

CXCL3 has chemotactic activity for neutrophils and may play a role in inflammation and exert its effect on endothelial cells in an autocrine fashion. In a recent study, upregulation of CXCL3 gene was observed by microarray experiment in crossbred during early hour of infection (Amod *et al.*, unpublished data). Similarly, Jensen *et al.* (2008) found the upregulation (fold difference 7.7,  $p < 0.05$ ) of CXCL3 gene in Sahiwal as compared to Holstein Friesian in resting monocytes at zero hour study while it showed downregulation at 2h (fold difference -1.5) and 72h (fold difference -1.2). Another chemokine Chemokine (C-X-C motif) ligand 2 also exhibited the same pattern of regulation at zero hour (fold difference 9.4), 2h (fold difference -1.5) and 72h (fold difference -1.1) in resting monocytes (Jensen *et al.*, 2008). In a study related to macrophage activation by LPS & IFN- $\gamma$  for the development of bovine macrophage specific cDNA microarray, CXCL3 showed 18.16 fold difference at 2h incubation and 15.28 fold difference at 16h of incubation (Jensen *et al.*, 2006). The varying susceptibility to tropical theileriosis, exhibited by different cattle breeds may be due to breed-specific differences in interaction of infected cells with other immune cells. These studies reveal that CXCL3 gene may have a role in breed specific resistance to *T. annulata* infection in early hours of infection.

CXCL3 gene was differentially regulated during *T. annulata* infection in early stage of infection ( $p < 0.05$ , fold change 2.53). However, there is a need to expand the investigation of host-pathogen interactions during *T. annulata* infection at different time intervals for CXCL3. Gene interaction network and its validation at protein level may further help in finding out the different gene functions during tropical theileriosis.

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