Mycobacterium Avium Subsp. Paratuberculosis Infected Cows Show Increased Expression of Toll-like Receptor 2 and 4(TLR2 And TLR4) in Their Peripheral Blood Mononuclear Cells (PBMCs)

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Background & Objectives: Mycobacterium avium subsp. paratuberculosis (M. ptb) is the causative agent of Johne's disease, a chronic granulomatous enteritis in ruminants, characterized by thickening of the intestinal wall and progressive wasting leading to death of the animal. Current estimates indicate that Johne's disease is one of the most costly infectious diseases of livestock in many countries including Iran. The immune response to mycobacterial infection involves phagocytosis of organisms by mononuclear phagocytes and sequestration within phagosomes. The capacity of the organism to prevent macrophage activation and phagosome maturation and to attenuate induction of a Th1 immune response appears to largely determine its pathogenicity. Our current understanding of the biology of M. ptb infection has been hindered by limited knowledge of the host factors involved in the immune response to the organism and the lack of appropriate molecular tools to dissect the host–pathogen interaction. Recognition by the host of molecular patterns displayed by microbes is essential to development of an effective immune response. Pattern recognition receptors (PRRs) are the main sensors of pathogen and danger signals in innate immunity. They are mainly expressed by macrophages and dendritic cells of different organs. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMPs). Recent studies have focused on the role of the TLRs family of cell membrane receptors in initiating cell signaling associated with mycobacterial infections. Activation of TLRs has also been shown to coordinate the adaptive immune response to microorganisms.

Methods: In this study, we aimed on the expression levels of bovine TLR2 and TLR4 transcripts in the peripheral blood mononuclear cells (PBMC) of cows infected with M.ptb by Real-time quantitative PCR (qPCR). Blood samples were taken from five cows which were positive in two consecutive ELISA test for paratuberculosis. As for the control, blood samples of five ELISA negative cows were also taken. After isolation of peripheral blood mononuclear cells (PBMC), total RNA was isolated and cDNA was synthesized using Oligo dT primers. Then, the primer pairs for TLR2, TLR4 as target genes and GAPDH and β-actin as housekeeping and calibrator genes were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression

levels of TLR2 and TLR4 transcripts in PBMC of paratuberculosis positive and negative cows.

Results: Statistical analysis using one way T-test showed a highly significant up-regulation of TLR2 and TLR4 expression in PBMC of paratuberculosis infected cows as compared to the negative control group ($p<0.001$).

Conclusion: the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of paratubeculosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.

Keywords: Gene Expression; Toll Like Receptors(TLRs); QPCR; Paratuberculosis