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Cytokines expression profile of *Brucella abortus* infected Indian water buffaloes

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ABSTRACT

In the present study investigation was carried out on the expression of cytokines in buffaloes infected with *Brucella abortus*. The study included, groups 1 and 2 of buffaloes each containing 5 of *Brucella* sero-positive buffaloes with history of abortions and sero-positive with no history of abortions respectively. Group 3, comprised 3 *Brucella* sero-negative buffaloes without history of abortion, and was considered as negative control. The transcript levels of IFN-γ, TNF-α, IL-2, IL-8 and IL-12 were analyzed using Taq-Man real-time polymerase chain reaction in blood cells of all animals. While comparing the levels of cytokines among these groups of animals, only TNF-α and IL-12 transcripts showed a significant ($P< 0.05$) decrease in animals of group 1 as compared to group 2. However, no significant difference was recorded in the levels of expression of remaining cytokines among the groups. These findings are perplexing and warrants an in depth analysis with large number of samples, to elucidate the complex interaction of various cytokines in immune response due to natural *Brucella* infection in Indian water buffaloes.

Key words: *Brucella abortus,* Buffaloes, Cytokines, Real-time TaqMan PCR

Bovine brucellosis caused by *Brucella abortus,* is one of the important zoonotic diseases worldwide particularly in developing countries including India (Trangadia *et al.* 2010). The causative agent, a facultative intracellular pathogens, infects host macrophages and only a few animals that become infected develop clinical signs (spontaneous abortion). Even in heavily *Brucella* infected buffalo herd, few animals remain negative and presumably non-infected all the time. This observation suggests that genetic variation within the host may play a role in resistance to brucellosis. In cattle, it is known that the resistance to brucellosis is genetically determined (Price *et al*. 1990). Cytokines are key molecules that play a major role in determining the protective immune response to a wide variety of infectious diseases. Therefore, the present study was undertaken with an aim to analyze the levels of various cytokines in *Brucella* infected and uninfected buffaloes.

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MATERIALS AND METHODS

Animals and blood samples: Blood samples (13) were collected from water buffaloes *(Bubalus bubalis*; breed Murrah) reared in an organized farm, where the presence of *Brucella spp.* was confirmed by isolation (Trangadia *et al*. 2010). However, the animals were apparently free from other infectious diseases. Groups 1 and 2 contain 5 each of seropositive buffaloes with history of abortions and sero-positive without history of abortions respectively. Whereas, group 3 with 3 sero-negative buffaloes with no history of abortion was considered as negative control.

Serological test: Detection of *Brucella abortus* antibodies in serum was carried out using an indirect ELISA kit.

RNA extraction and real-time PCR: Total RNA (tRNA) from blood was isolated using kit according to the manufacturer's instructions. Quality of RNA samples was determined by the presence of at least 2 intact bands of 28S and 18S on a formamide treated ethidium bromide stained 1% agarose gel. cDNA synthesis was performed immediately following RNA extraction using kit according to manufacturer's protocol and stored at –20° C till further use. In the absence of specific primer and probe information for cytokines in buffaloes, bovine sequences described in previous report (Leutenegger *et al.* 2000) were utilized with

certain modifications for real-time assay of gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-2 (Interleukin-2), IL-8 and IL-12. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as internal control. The real time PCR was performed using TaqMan probes labeled with FAM and TAMRA.

Each cDNA was assayed for different cytokine expression and for GAPDH as an endogenous control in separate wells of 96 well optical plates (ABI) in a 25 µl PCR mix. The PCR mix contained 400 nM primers and 80 nM TaqMan probes and TaqMan universal PCR master mix (ABI). The amplification was performed on real time PCR system (ABI) under following conditions: 2 min at 50°C, 10 min at 95° C, 40 cycles of 15 sec at 95°C and 60 sec at 60° C. Quantification of cytokines expression was done according to the comparative threshold cycle (C_T) method using ABI 7300 System sequence detection software version 1.3.1 keeping the cytokine with minimum transcript level as calibrator.

Statistical analysis: Statistical analysis between groups for individual cytokine was performed with two-sample independent t-test by using Origin Pro 7.5 SR4 analysis software. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

In the present study, the transcript levels of IFN-γ, TNF-α, IL-2, IL-8 and IL-12 were analyzed in blood samples of 3 groups of buffaloes. While comparing the levels of cytokines between groups of animals, only TNF-α and IL-12 transcripts showed a significant decrease ($P < 0.05$) in the buffaloes of group 1 as compared to group 2. However, no significant difference was observed among the buffaloes of groups 2 and 3 (Fig. 1).

IFN-γ and TNF-α are proinflammatory cytokines required for generating macrophages with strong activity in killing intracellular bacteria. TNF-α acts also as co-stimulant of IFN-γ production (Odbileg *et al.* 2008). Moreover, IL-12 stimulates differentiation of Th 0 into Th1 helper cells, which produces IFN-γ. Cytokine IFN-γ is responsible for macrophage activation and restriction of *Brucella* infection *in vitro* and *in vivo*. It is hypothesized that, in this study the low transcripts of TNF-α and IL-12 with no significantly different IFN-γ level in aborted animals (group 1) may not be able to produce the optimum level of IFN-γ required for control of intracellular bacterial multiplication and that might have resulted in abortion as compared to group 2. A significant decrease in TNF-α level in *Brucella* infected camels compared to uninfected was reported by El-Boshy *et al.* (2009).

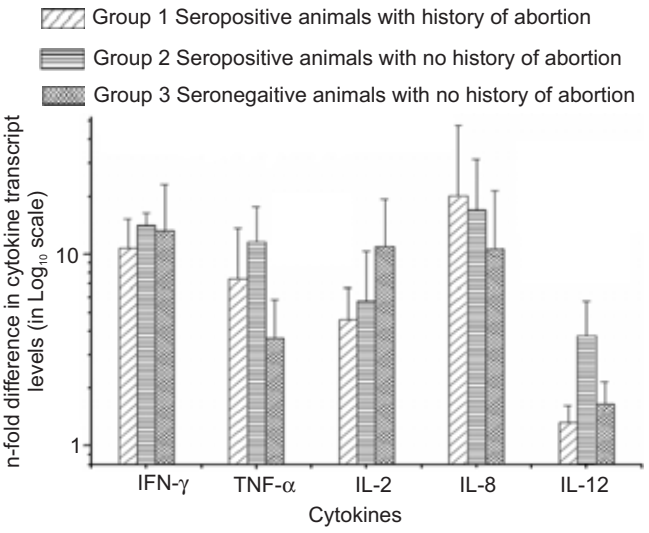

Fig 1. Cytokine expression profile in blood cells of *Brucella abortus* infected buffaloes.

No significant difference was also recorded in the level of IL-2 between any of the group of animals. Similar results were reported by Splitter *et al.* (1996) in cattle. There was no significant difference in the level of IL-8 between any of the groups under study. Akbulut *et al.* (2007) also observed no significant difference in level of IL-8 in human patients with brucellosis and control groups.

Although it was documented that cytokines may provide protective immunity to brucellosis, it is suggested that optimal development and maintenance of a protective response against infection depends on a finely regulated balance of cytokines, rather than upon the level of a single cytokine. From the results of the present study no clear correlation could be established on the extend of cytokine expression and the level/stage of *Brucella* infection. A detailed study on a larger sample size with several cytokines in infected and uninfected buffaloes would be required for better understanding of their complex interaction in immune response to brucellosis.

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