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Serum interferon-gamma and interleukins-6 and -8 during infection with *Fasciola gigantica* in cattle and buffaloes

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This study investigated the presence of cytokines interferon (IFN)-gamma, interleukins (IL) -6 and -8 in serum of cattle and buffaloes infected with *Fasciola gigantica* from one to 16 weeks post-infection to determine their T cell response during infection. The concentration of these cytokines was determined by sandwich enzyme-linked immunosorbent assay (ELISA). No IFN-gamma was detected in these animals while IL-6 was elevated from one to 16 weeks post-infection. Levels of IL-8 were also elevated in infected buffaloes from one to 16 weeks post-infection. A predominantly T helper (Th) 2 response which started early in the infection was apparently present in cattle and buffaloes in this study which was characterised by IL-6. IL-8 production could be another mechanism of immune response in buffaloes during infection with *F. gigantica*.

Key words: buffaloes, cattle, cytokines, *Fasciola gigantica*

Introduction

Fasciola gigantica is a common parasite of cattle and buffaloes in the tropics and causes significant economic losses to agricultural and livestock production [26,28]. Despite the importance of tropical fasciolosis, information on the nature of the immune response induced during infection is limited. Generally, helminth infections are manifested by suppression of T helper (Th) 1 function and induction of T cells which express cytokines characteristic of the Th2 subset [5,10] Studies with *F. hepatica* in cattle and sheep have demonstrated that the T cell response is polarized towards a type 2 response [5,6,17,18,29]. There is no published information regarding cytokine profiles during *F. gigantica* infection hence information on T cell response during infection is lacking. This study was undertaken to investigate cytokine production in cattle and buffaloes infected with *F. gigantica* to give an indication of the T cell response and provide a basis of understanding host-parasite relationship during *F. gigantica* infection in these animals.

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Materials and Methods

Experimental animals and their maintenance

Sixteen cattle, 7-10 months of age, were purchased from a ranch in Kiblawan, Davao del Sur, Mindanao, Philippines. Sixteen buffaloes, aged 7-12 months, were purchased from farmers in Cotabato Province, Philippines. At purchase, animals were free of detectable eggs of *F. gigantica* in their faeces. They were treated with triclabendazole (Fasinex 240; Novartis, Switzerland) and ivermectin (Ivomec; Merial, UK) and allocated at random into infected [8] and control [8] groups for each species. The animals were maintained in pens on a diet of freshly cut napier grass *ad libitum* and 2 kg of grain concentrate per animal per day. Mineral lick and water were also provided *ad libitum*. The animals were cared for in compliance with the Australian Code of Practice for the Care and use of Animals for Scientific Purposes.

Infection with *F. gigantica*

After an acclimatisation period of two weeks, animals were infected with a single dose of 1000 viable metacercariae of *F. gigantica*. The metacercariae were obtained from infected *Lymnaea rubiginosa* collected at Midsayap, Cotabato Province, Philippines. Metacercariae were administered within 1 week of harvesting by oral infection on a bolus of filter paper.

Blood collection

Blood was collected from the jugular vein once weekly for 16 weeks. Serum obtained from clotted blood after centrifugation was kept at –20°C until analysis.

Cytokine analysis

Levels of IFN-gamma (γ) in serum of cattle and buffaloes were assessed using a solid phase sandwich enzyme immunoassay kit (Bovine γ Interferon Test; CSL Biosciences, Australia). The levels of IL-6 and IL-8 in serum of infected

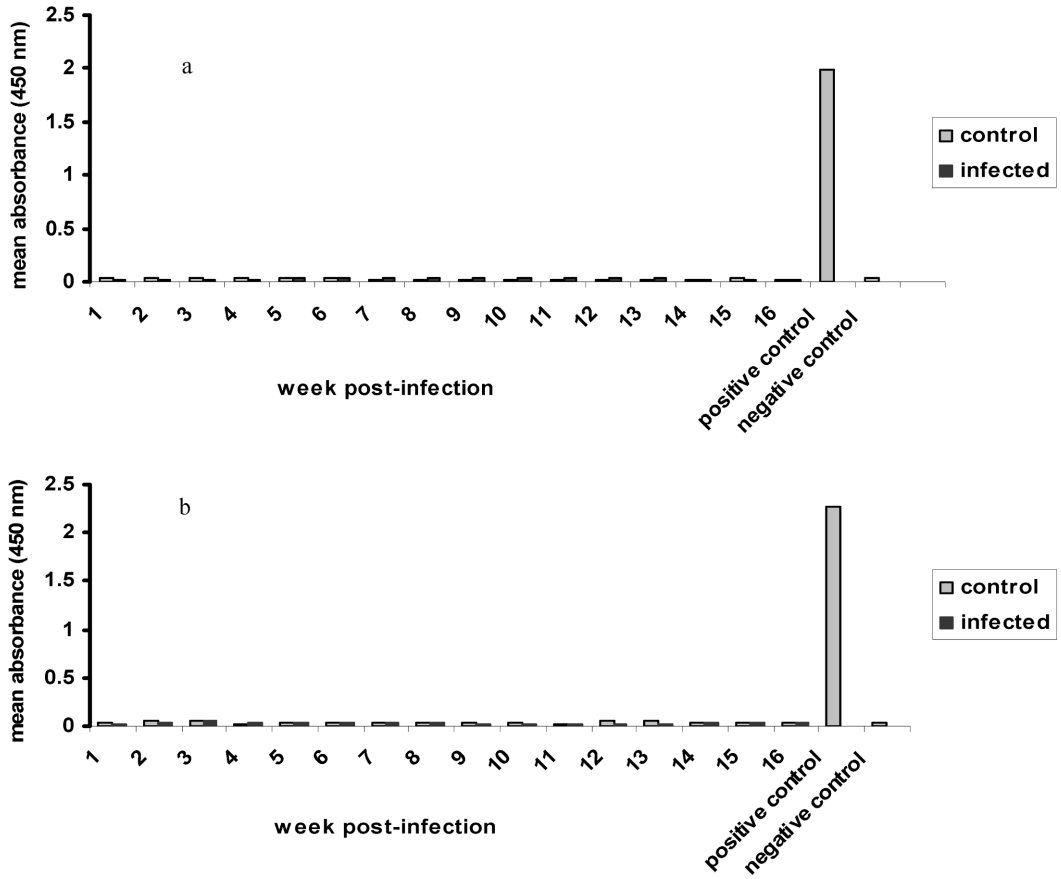

Fig. 1. IFN-γ profile in cattle (a) and buffaloes (b) infected with *F. gigantica.*

and non-infected cattle and buffaloes were determined by ELISA. The assay made use of mouse anti-ovine IL-6 or IL-8 at 5 μg/ml as the coating antibody and rabbit anti-ovine IL-6 (Center of Animal Biotechnology, University of Melbourne and Epitope Technologies, Australia) or IL-8 (Epitope Technologies, Australia) diluted at 1 : 5000 as the detector antibody. Conjugate used was anti-rabbit Ig-HRP (Tropbio; James Cook University, Australia) diluted at 1 : 120. Tetramethylbenzidine (TMB) substrate solution was used as the enzyme substrate. Conjugate controls were included in each plate. Recombinant ovine IL-6 and IL-8 (DPI; Geelong, Australia) were used as the positive controls. Absorbance was obtained at 450 nm using an ELISA plate reader and background readings were subtracted from readings of the unknown samples. Values obtained were read against the standard curve taking into consideration the dilution factor.

Results

IFN-γ production

No IFN-γ production was observed in infected and control cattle and buffaloes from 1 to 16 weeks post-infection (Fig. 1).

Serum IL-6 and IL-8 levels

Levels of serum IL-6 were increased in cattle and buffaloes infected with *F. gigantica* (Fig. 2). Serum IL-8 levels were higher in infected buffaloes compared to levels in control buffaloes while IL-8 levels in infected cattle were lower than control cattle (Fig. 3).

Discussion

The present study is the first to investigate levels of IFN-γ, IL-6 and IL-8 during infection with *F. gigantica*. Results show that the T cell response of cattle and buffaloes infected with *F. gigantica* in this study was apparently a type 2 response, with a downregulation of a Th1 response. This is indicated by an absence of IFN-γ production and the presence of IL-6 from one to 16 weeks post-infection in these animals indicating that the Th2 response commenced early and persisted throughout the 16-week observation period. IL-6 is one of the cytokines produced by Th2 cells [1,7] and it participates in the polarization of the immune response towards a Th2 response [2]. A similar result was reported by Clery *et al*. [5] who did not detect IFN-γ in cattle during a chronic infection with *F. hepatica*. A predominant

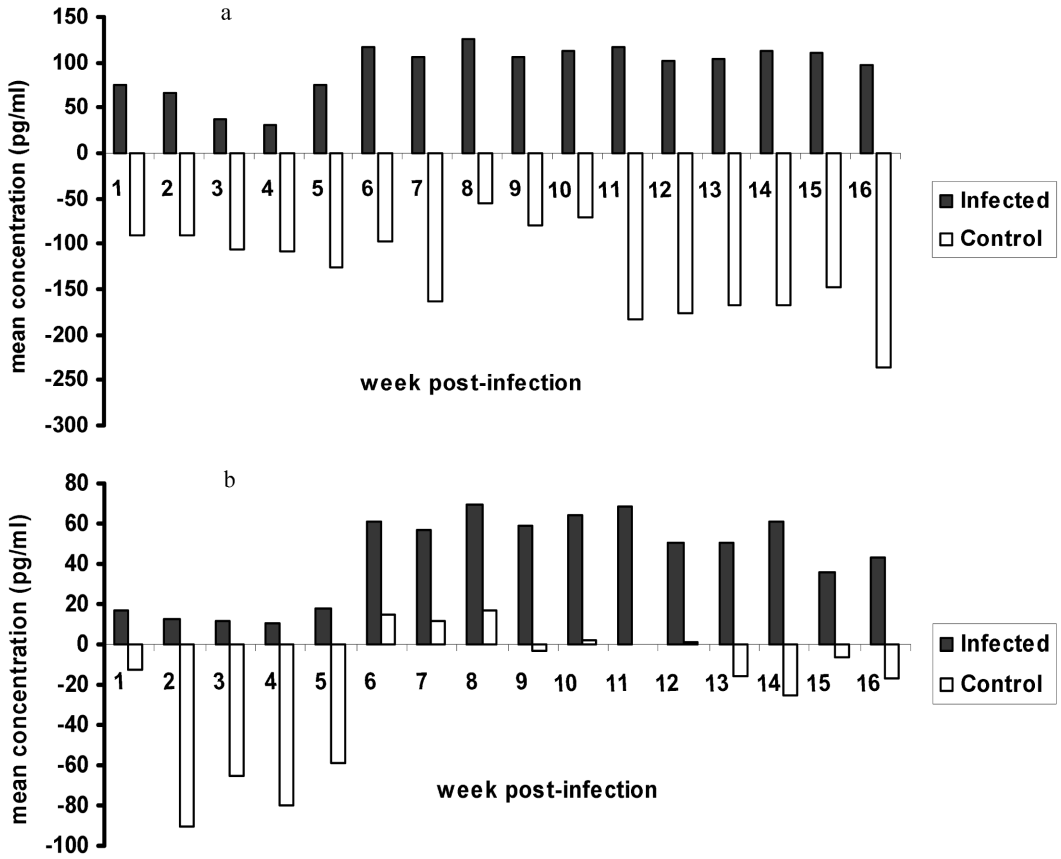

Fig. 2. Serum IL-6 levels in cattle (a) and buffaloes (b) infected with *F. gigantica*.

Th2 response has been reported in rats, sheep and cattle infected with *F. hepatica* [19]. More recently, Waldvogel *et al*. [29] observed that peripheral blood mononuclear cells of calves experimentally infected with *F. hepatica* expressed high amounts of IL-4 but not of IFN-γ mRNA early in the infection indicating a Th2 biased immune response commencing early in the infection. The IgG1, IgE and eosinophilia are features associated with a Th2 response [8,24]. Clery *et al*. [5] observed that IgG1 was the dominant isotype present in cattle infected with *F. hepatica* in their study, with IgG2 occurring at much lower levels. The IFN-γ response that commenced early in the infected cattle and buffaloes in this study may have inhibited their Th1 production.

The increased serum IL-6 in infected cattle and buffaloes and increased IL-8 in infected buffaloes suggests that these cytokines may have a role in the immune reaction during liver fluke infection in some species. These cytokines were demonstrated in humans infected with *F. hepatica* [15] but there is no published information regarding their role in the immunity during liver fluke infection.

IL-6 and IL-8 are both involved in an antibody-dependent cell-mediated cytoxicity (ADCC) involving neutrophils as shown by a number of studies [3,4,11,12,16,25]. It was demonstrated that IL-6 inhibited hepatic stages of *Plasmodium* through an oxidative burst [21] and primed neutrophils' ability to kill *Salmonella typhimurium* [20]. IL-8 also enhances the phagocytic ability of neutrophils during the immune and inflammatory responses to pathogens [12,16]. In fasciolosis, ADCC has been considered to be a mechanism by which flukes are destroyed. In *F. hepatica*-resistant rats larvae of *F. hepatica* were coated with antibody and host cells, including eosinophils, neutrophils, macrophages and mast cells, before they were destroyed within the peritoneal cavity [14]. Hansen *et al*. [13] suggested that killing of flukes in the *F. gigantica*-resistant Indonesian thin-tailed (ITT) sheep may be due to an ADCC reaction, a mechanism also supported by Estuningsih *et al*. [9] who observed that macrophages of ITT sheep demonstrated an ADCC against *F. gigantica*. The mechanism of killing juvenile flukes in *F. hepatica*-resistant rats was identified as the release of high levels of nitric oxide by peritoneal lavage cells [22,23,27]. Cattle and buffaloes, by producing IL-6 and IL-8 (in buffaloes) during infection with *F. gigantica*, may thus be capable of exerting a cytotoxic effect against the fluke.

In conclusion, cattle and buffaloes infected with *F. gigantica* in this study had a predominant Th2 response which started early in the infection. IL-6 production in these

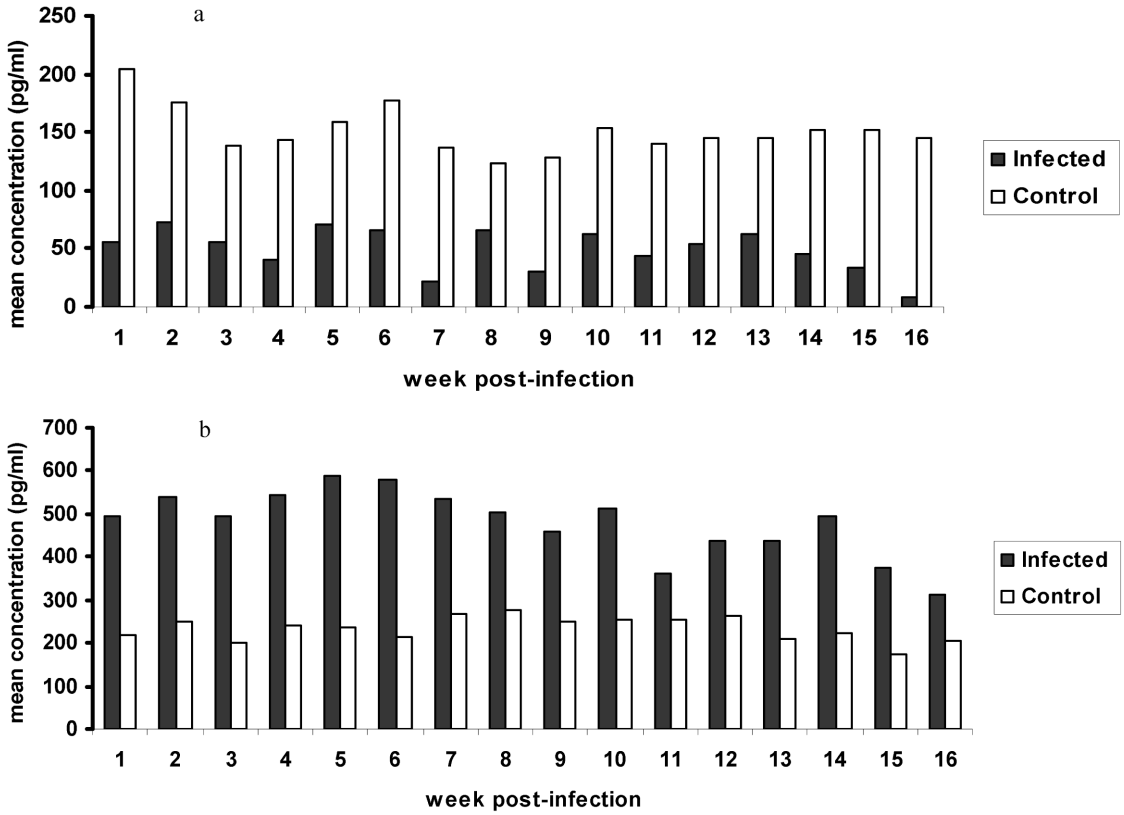

Fig. 3. Serum IL-8 levels in cattle (a) and buffaloes (b) infected with *F. gigantica*.

animals apparently influenced the initiation and maintenance of a type 2 immune response thereby down-regulating Th1 response. IL-6 and IL-8 (in buffaloes) may be involved in a cytotoxic mechanism in cattle and buffaloes against *F. gigantica*. In addition, immunity to *F. gigantica* differs between cattle and buffaloes, with the latter capable of producing IL-8 during infection.

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