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Clinical, haematological, cytokine and acute phase protein changes during experimental *Babesia gibsoni* infection of beagle puppies

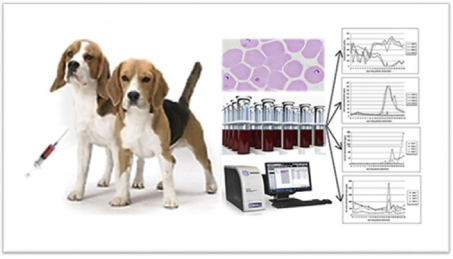
A.L. Brown [a], R.E. Shiel [b], P.J. Irwin [a, *]

[a] Murdoch University Veterinary Hospital, College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, South Street, Murdoch, Perth, Western Australia, 6150, Australia
[b] University College Dublin Veterinary Hospital, Veterinary Science Centre, School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

HIGHLIGHTS

- Experimental *B. gibsoni* infection caused transient pyrexia and anaemia.
- Infection induced a transient thrombocytopenia without apparent clinical effect.
- The acute phase response was marked but delayed and preceded parasitaemia.
- *B. gibsoni* infection induced marked but delayed increases in multiple cytokines.
- Cytokine alterations occurred following the acute phase response and parasitaemia.

GRAPHICAL ABSTRACT



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ABSTRACT

Babesia gibsoni is a haemoprotozoan parasite of emerging global importance. The clinical presentation of babesial infections is diverse and the systemic inflammatory response induced by infection is considered to be a major feature of the pathophysiology of canine babesiosis. An experimental case-controlled longitudinal study was conducted to assess the clinical, haematological, cytokine and acute phase protein changes that occur during experimental *B. gibsoni* infection of beagle puppies. Infected dogs became transiently pyrexic and anaemic, intermittently neutropenic and transiently, but profoundly, thrombocytopenic, although this had no apparent adverse clinical effect. Experimental *B. gibsoni* infection also induced an acute phase response, characterised by a marked increase in the concentration of C-reactive protein, which was delayed in onset following infection but preceded the detection of peripheral parasitaemia. Experimental *B. gibsoni* infection was also associated with marked increases in the concentration of multiple cytokines which were also delayed in onset following infection and occurred subsequent to the detection of peripheral parasitaemia and the acute phase response. This study furthers our understanding of the immune response that occurs during babesial infections and the role that systemic inflammation plays in the pathophysiology of canine babesiosis.

* Corresponding author.
E-mail address: p.irwin@murdoch.edu.au (P.J. Irwin).

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1. Introduction

Babesia gibsoni is a vector-borne haemoprotozoan parasite of emerging global importance with infections documented throughout Asia, Australia, Africa, North and South America and Europe (Irwin, 2009). Infection occurs following the bite of an infected ixodid tick from the genus *Haemaphysalis* and, possibly, the genus *Rhipicephalus* (Irwin, 2010). Sporozoite transmission into the bloodstream of the canine host results in erythrocyte invasion, intra-erythrocytic multiplication, and subsequent erythrocyte lysis, releasing more parasites into the bloodstream to infect additional erythrocytes or new ixodid vectors (Irwin, 2010). Non-vectorial routes of transmission have also been documented for *B. gibsoni* including transplacental transmission and transmission by blood transfusions or fighting (Stegeman et al., 2003; Fukumoto et al., 2005; Jefferies et al., 2007a).

The clinical presentation of canine babesiosis can vary widely from subclinical to fulminant disease resulting in multiple organ failure and death (Irwin, 2010). The severity of the disease is primarily determined by the species of *Babesia* parasite involved, with *B. gibsoni* considered intermediate in its pathogenicity (Schoeman, 2009). However, host factors, including age and immune response, can also play a role in determining the severity of the disease (Oduye and Dipeolu, 1976; Lewis et al., 1995; Jacobson, 2006). Natural and experimental *B. gibsoni* infections are typically characterised by pyrexia, lethargy, anaemia, thrombocytopenia, icterus and splenomegaly (Jefferies et al., 2007b; Schoeman, 2009).

Infection with babesial parasites induces a systemic inflammatory response in the host and this is considered to be a major feature of the pathophysiology of canine babesiosis which contributes to its diverse clinical manifestations (Welzl et al., 2001; Matijatko et al., 2007; Koster et al., 2009; Schetters et al., 2009). Cytokines play a critical role in the initiation and development of systemic inflammation and are responsible for mediating and regulating all aspects of the immune response to infection (Borghetti et al., 2009; Lewis et al., 2012). However, while important for host defence, excessive production and release of these immunoregulatory mediators can prove deleterious to the host, initiating widespread tissue injury and organ damage (Borghetti et al., 2009; Lewis et al., 2012). Longitudinal kinetic profiling of a broad range of cytokine alterations that occur during an infection can be useful to expand our understanding of the immunopathogenesis of infectious diseases and host-adaptive humoral and cell-mediated immune responses to infection, as well as potentially allowing the identification of future diagnostic markers and therapeutic interventions.

The immunopathogenesis of canine babesiosis is poorly understood at a cellular level. To date, the only cytokine investigated in canine babesiosis is tumour necrosis factor alpha (TNF-α) which was reported in a single study that assessed TNF-α concentrations in dogs naturally infected with *Babesia rossi* at the time of presentation to a veterinary hospital with clinical signs of babesiosis (Vaughan-Scott, 2001). In this study higher TNF-α concentrations were found in dogs with higher peripheral parasitaemias and more severe disease, although no association between TNF-α concentration and mortality was identified. The kinetics that occur in other cytokines in canine babesiosis have yet to be characterised.

In response to inflammatory cytokine secretion, increased production of positive acute phase proteins, such as C-reactive protein (CRP), occurs as part of the innate immune response to infection (Murata et al., 2004; Ceron et al., 2005). In dogs, CRP is regarded as a major acute phase protein and can be used as a sensitive biomarker to quantify systemic inflammation (Murata et al., 2004; Ceron et al., 2005). An acute phase response to natural *B. rossi* infections, and both natural and experimental *Babesia canis* infections, has been documented previously (Ulutas et al., 2005; Matijatko et al., 2007; Koster et al., 2009; Schetters et al., 2009). Increased concentrations of CRP were identified in dogs naturally infected with *B. rossi* and *B. canis* at the time of presentation to a veterinary hospital with clinical signs of canine babesiosis and persistently increased CRP concentrations were noted in dogs within 2–4 days of experimental infection with *B. canis* (Ulutas et al., 2005; Matijatko et al., 2007; Koster et al., 2009; Schetters et al., 2009). However, no association between CRP concentration and disease outcome was identified. The acute phase response to *B. gibsoni* infection has yet to be characterised.

The aim of this longitudinal study was to investigate the cytokine kinetics together with the clinical, haematological and acute phase protein changes that occur during experimental *B. gibsoni* infection of beagle puppies.

2. Materials and methods

2.1. Experimental animals

Four intact five month old beagle litter-mates were used in an experiment performed as part of the production of microscope slides used commercially in an indirect fluorescent antibody test (IFAT) for the diagnosis of *B. gibsoni* infections. All four dogs were confirmed to be free of *B. gibsoni* infection by polymerase chain reaction (PCR) and IFAT (Vetpath Laboratory Services (VLS), Australia) prior to the start of the experiment. All four dogs were housed together in the Animal House facility at Murdoch University for the duration of the experiment and managed under identical conditions. The experiment was started after an acclimatisation period of two weeks. All four dogs were fed a commercial dry dog food (Supercoat Puppy, Purina) twice daily and provided with *ad libitum* access to water. The study was approved by the Murdoch University Animal Ethics Committee (Permit Number: R2442/11).

2.2. Experimental B. gibsoni infection

Blood was collected from an American pit bull terrier (red blood cell count 5.2×10^{12}/L; reference interval $5.5–8.5 \times 10^{12}$/L) suspected to be infected with *B. gibsoni*. Parasites were not observed in erythrocytes on manual blood smear evaluation but infection was confirmed by nested PCR amplification of a partial fragment of the 18S rRNA gene of *B. gibsoni* as described previously (Jefferies et al., 2007a). Dogs 1 and 2 were infected with *B. gibsoni* (Day 0) via intravenous injection of 10 ml of ethylenediaminetetraacetic acid (EDTA) anti-coagulated whole blood collected from the American pit bull terrier. Dogs 3 and 4 were used as situational controls. The experiment was terminated on Day 23 to allow production of the IFAT slides. Infected dogs were humanely euthanased by barbiturate overdose on Day 24.

2.3. Clinical evaluation

A complete physical examination was performed on Days 0–23 inclusive in all dogs. Heart rate, respiratory rate and body temperature were recorded. Pyrexia was defined as a body temperature greater than 39.3 °C.

2.4. Sample collection

Blood (1.3–9.3 ml) was collected from each dog every day by jugular venepuncture and placed into potassium EDTA (Sarstedt, Australia) (1.3 ml), lithium heparin (Greiner Bio-One, United States of America) (4 ml) and serum (Greiner Bio-One, United States of America) (4 ml) tubes. Samples were centrifuged within 2 h of