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Evidence of an acute phase response in dogs naturally infected with *Babesia canis*

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Abstract

The erythrocyte sedimentation rate (ESR), white blood cell count (WBC), haematocrit (HCT) and platelet number (PLT) were quantified and compared with the acute phase proteins (APPs) in dogs naturally infected with *Babesia canis* and healthy dogs. Both groups were treated with imidocarb dipropionate on the day of admission and both groups were monitored for all parameters on the admission day and on the first, second, third, fourth and seventh days in order to determine the presence of an acute phase reaction, to assess the diagnostic value of these markers in uncomplicated canine babesiosis and to evaluate the use of APPs in treatment monitoring.

It was demonstrated that an acute phase response occurs in dogs naturally infected with *Babesia canis*, with significant increases in the concentration of major acute phase proteins. The serum concentration of C-reactive protein (CRP), serum amyloid A (SAA) and the erythrocyte sedimentation rate (ESR) decreased daily after treatment and approached reference range values by the eighth day. PLT and haematocrit (HCT) increased daily after treatment and approached reference range values by the fourth day. WBC and haptoglobin increased after treatment and then decreased from the third and fourth days, respectively, to the eighth day. The diagnostic sensitivity of CRP, SAA and PLT was significantly higher compared to haptoglobin, ESR, HCT and the WBC count. CRP and SAA were of clinical use in monitoring the response to antibabesial treatment.

Keywords: *Babesia canis*; Acute phase reaction; Pathology; Treatment; Imidocarb dipropionate; C-reactive protein; Serum amyloid A; Haptoglobin

1. Introduction

Canine babesiosis is a tick-borne disease caused by the intra-erythrocytic protozoan parasites *Babesia canis*, *Babesia gibsoni* and *Babesia microti*-like piroplasms (Uilenberg et al., 1989; Taboada and Merchant, 1991; Camacho et al., 2001). Previous studies have suggested that *B. canis* should be divided into three biologically and immunologically distinct subspecies: *B. canis canis*, *B. canis vogeli* and *B. canis rossi* (Uilenberg et al., 1989). However, additional molecular biological studies have indicated that these three groups of parasites do not

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cluster in a single clade, which suggests that they are not subspecies (Carret et al., 1999). Since the discussion about classification of the *Babesia* species is not the aim of this paper, the classical nomenclature (*B. canis*, *B. rossi* and *B. vogeli*) will be used as suggested by Schetters et al. (1997), Zahler et al. (1998), Carret et al. (1999), Passos et al. (2005), and Schetters (2005). Canine babesiosis caused by *B. canis* is a very common cause of morbidity and mortality of dogs in Croatia (Caccio et al., 2002). The disease can be clinically classified into uncomplicated and complicated forms. Dogs with uncomplicated babesiosis are typically presented with signs relating to acute haemolysis, including pale mucous membranes, fever, anorexia, depression, splenomegaly and water hammer pulse (Taboada and Merchant, 1991). The clinical manifestation of the complicated form is variable and related to the complications developed. The complications of canine babesiosis are acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, immune-mediated haemolytic anaemia (IMHA), peracute babesiosis, acute respiratory distress syndrome (ARDS), haemoconcentration and shock (Lobetti, 2000).

The clinical presentation of canine babesiosis caused by *B. canis* is highly variable. The many and varied clinical manifestations of this disease are difficult to relate to an organism that is solely restricted to the erythrocytes. Some authors have proposed that although the clinical manifestations are diverse, the mechanism promoting them is probably uniform (Jacobson and Clark, 1994; Lobetti, 1998; Welzl et al., 2001). They consider systemic inflammation as a major feature in the pathophysiologic mechanisms of this disease and have suggested that systemic inflammatory response syndrome (SIRS) and subsequent multiple organ dysfunction syndrome (MODS) provide the underlying pathophysiologic mechanism within which apparently unrelated aspects of babesiosis form a predictable pattern.

Acute phase reaction develops following any tissue inflammatory injury and is considered as a part of innate immunity characterized by profound changes in the concentration of acute phase proteins (APP) in the plasma (Moshage, 1997; Gabay and Kushner, 1999). The circulating concentration of acute phase proteins is related to the severity of the underlying condition and it provides a means for evaluating the presence and extent of the disease process as well as the efficacy of the disease management in human and veterinary medicine (Steel and Whitehead, 1994; Pannen and Robotham, 1995; Gruys et al., 1994; Martinez-Subiela et al., 2002, 2003).

The acute phase reaction in naturally occurrring canine babesiosis has been poorly described. Lobetti et al. (2000) documented a marked acute phase reaction by the detection of increased concentrations of α1-acid glycoprotein in canine babesiosis caused by *B. rossi*, and Matijatko et al. (2002) described a significant increase of CRP in naturally occurring canine babesiosis. Moreover, to the authors' knowledge no studies about the use of acute phase proteins to monitor the response to antibabesial treatment have been published. Additionally, although it has been demonstrated that haptoglobin and ceruloplasmin have higher diagnostic sensitivity than WBC counts to detect inflammation in dogs (Solter et al., 1991), there is a lack of studies in which the diagnostic sensitivity of various traditional markers of inflammation or sepsis (such as ESR, total and differential WBC counts or platelet counts), and various major (CRP and SAA) and moderate (haptoglobin) acute phase proteins are evaluated and compared.

In this study, therefore, serum concentrations of C-reactive protein, serum amyloid A and haptoglobin were investigated in dogs naturally infected with *B. canis* in order to document the acute phase reaction and its potential value in the diagnosis of the disease. In addition, the use of the sequential measurement of acute phase proteins in assessing the response to antibabesial treatment was also evaluated. During the entire study, acute phase proteins were compared to commonly used inflammatory markers such as the erythrocyte sedimentation rate, WBC count and platelet count. Haematocrit determinations were included in our work in order to quantify the degree of anaemia and better understand the pathogenesis of the disease.

2. Materials and methods

2.1. Animals

The study was performed on two groups of animals.

Group 1 consisted of 50 dogs naturally infected by *B. canis*, admitted to the Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia, with clinical signs of acute babesiosis. The diagnosis was confirmed by demonstration of the parasites within the infected erythrocytes in Romanowsky-stained thin blood smears. One dose (6 mg/kg) of imidocarb dipropionat (Imizol®, Shering-Plough) was administered to all the dogs subcutaneously on the day of admission. On the basis of clinical presentation, laboratory data and response to antibabesial treatment, only uncomplicated cases of babesiosis were included