Characterisation of serum protein electrophoresis patterns and C-reactive protein in canine tick-borne diseases

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Research Article

Keywords: blood parasites, C-reactive protein, dog, serum protein, electrophoresis

Posted Date: March 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-322609/v2>

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Abstract

Background

Canine tick-borne diseases are important diseases with a worldwide distribution. In Thailand, the most important canine tick-borne diseases are ehrlichiosis, babesiosis and hepatozoonosis. The aim of this study was to determine the serum protein electrophoresis patterns and C-reactive protein levels associated with *E. canis*, *B. canis* or *H. canis* single infections.

Results

In dogs infected with *E. canis*, *B. canis* and *H. canis* single infections, the albumin levels and A/G ratios were significantly decreased, whereas β 2-globulin levels were increased ($P < 0.05$). The γ -globulin level was significantly increased in *E. canis* and *H. canis* infections ($P < 0.05$). A monoclonal gammopathy pattern was observed in *E. canis* and *B. canis* single infections while β - γ bridging patterns and increased β - and γ -globulin fractions were found in *H. canis* single infections. The C-reactive protein level was increased in dogs with blood parasite single infections and may be related to the pathogenesis of the infection.

Conclusions

Serum protein electrophoresis patterns and C-reactive protein levels can be used to monitor health status and blood parasite problems in infected dogs.

Background

Canine tick-borne diseases are transmitted by *Rhipicephalus sanguineus* (brown dog tick), which is common in Thailand [1] and is distributed worldwide [2]. Ticks not only feed on blood cells but also transmit different types of pathogen, for example, protozoa, virus, rickettsia and bacteria [3], causing both morbidity and mortality. *Ehrlichia canis*, *Babesia canis vogeli* and *Hepatozoon canis* are commonly found in Thailand [4, 5]. Ehrlichiosis and babesiosis can develop when a dog is bitten by an infected tick, but hepatozoonosis is caused by its ingestion. *B. canis* subspecies *vogeli* is the main species causing canine babesiosis in Thailand [5]. *B. canis* is the large form of *Babesia* spp., and *Babesia* organisms enter and multiply in erythrocytes of the host. Clinical manifestations are anorexia, lethargy, pale mucous membranes, fever, jaundice, and renal disease [6]. The severity of infection depends on the subspecies of canine babesiosis. *B. canis* causes a sub-clinical-to-mild degree of infection [6, 7]. *E. canis* is a gram-negative intracellular rickettsia that can infect monocytes and lymphocytes in dogs [8]. Canine ehrlichiosis can be classified into three stages according to clinical signs: acute, sub-clinical and chronic. In the acute stage, clinical signs appear around 1–3 weeks after infection and include fever, weakness, lethargy, depression, lack of appetite and limb oedema. In the sub-clinical stage, the organism may be

present for months to years without clinical symptoms. In the chronic stage, the infected dog has abnormal bleeding due to thrombocytopenia, severe weight loss, fever, difficulty in breathing due to lung inflammation, joint pain, seizures in some cases, a lack of coordination, anaemia and kidney failure [9]. *H. canis* is an apicomplexan parasite that belongs to the family Hepatozoidae and clinical signs of *H. canis* infection can vary from sub-clinical to severe and life-threatening. The most frequently observed clinical signs are anaemia, extreme lethargy, intermittent fever and emaciation [7].

SPEPs show the fractions of two major types of protein: albumin and globulin. Albumin is a crucial protein component in the serum and is produced by the liver. Globulins account for a smaller fraction of total serum content. In dogs, globulin fractions can be separated into five fractions: α 1-globulin, α 2-globulin, β 1-globulin, β 2-globulin and γ -globulin. The measurement of serum protein may be key in the detection, diagnosis and monitoring of various diseases and pathological processes. SPEPs can be used as a diagnostic tool in a wide spectrum of diseases, including infectious and inflammatory disease, renal disorders, hepatic disorders, gastrointestinal disorders, immunodeficiency status and paraproteinemia caused by plasma cell neoplasia [10].

The acute-phase response is considered part of the innate host defence system and systemic effects include leukocytosis, fever and increased blood cortisol. C-reactive protein (CRP) is one of the acute-phase proteins and is synthesised by hepatocytes, smooth muscle cells, macrophages, endothelial cells, lymphocytes and adipocytes. It is a major acute-phase protein in dogs and is part of the γ -globulin fraction. Its concentration increases dramatically in response to inflammation, infection from pathogens (including bacteria and parasites), and injury, and it has been used as a predictive marker for risk of disease and to monitor the response to treatment [11]. The aim of this study was to investigate the SPEPs and CRP concentrations associated with single infections of *E. canis*, *B. canis* and *H. canis* and in canine blood parasite co-infection.

Results

Fifty-six serum samples were assessed for total protein content by biuret colorimetric test. The SPEPs was determined by agarose gel electrophoresis (Figs. 1). Tables 1 and 2 show the results of total protein, albumin and globulin fractions and A/G ratio in single blood parasite infections. Comparisons were made among single infections of *B. canis*, *E. canis* or *H. canis* in normal dogs and between groups. We found no significance differences between three groups of blood parasite infection. In this study, the monoclonal gammopathy was 39% (5/13) of *B. canis* and 35% (7/20) of *E. canis* single infections. In dogs with *H. canis* single infection, the serum protein pattern showed β - γ bridging and an increase in the β - and γ -globulin peaks in 43% (6/14) of cases.

Table 1

The results of relative concentration of serum protein fraction (%) and albumin/globulin (A/G) ratio in normal dogs and dogs infected with *B.canis*, *E.canis* or *H.canis* single infections (mean $\pm$ SD). *P*-value – significance of the analysis of variance; n.s.: not significant. *Significant difference between normal and *B.canis*, *E.canis* or *H.canis* at *P* < 0.05.

Variables	Normal (n = 9)	<i>B. canis</i> (n = 13)	<i>E. canis</i> (n = 20)	<i>H. canis</i> (n = 14)	<i>P</i> -value
Albumin	42.43 $\pm$ 6.29	32.38 $\pm$ 9.29*	27.18 $\pm$ 7.84*	23.15 $\pm$ 11.64*	< 0.05
α 1-globulin	6.02 $\pm$ 1.86	5.85 $\pm$ 2.96	5.19 $\pm$ 1.80	3.80 $\pm$ 1.72*	< 0.05
α 2-globulin	6.40 $\pm$ 3.95	8.35 $\pm$ 5.62	10.87 $\pm$ 5.78*	6.74 $\pm$ 5.42	< 0.05
β 1-globulin	10.40 $\pm$ 4.47	10.92 $\pm$ 5.30	10.20 $\pm$ 5.72	10.54 $\pm$ 6.81	n.s.
β 2-globulin	13.90 $\pm$ 4.30	18.89 $\pm$ 14.10*	20.81 $\pm$ 8.23*	20.11 $\pm$ 9.07*	< 0.05
γ -globulin	25.32 $\pm$ 8.71	23.57 $\pm$ 13.25	25.93 $\pm$ 9.87	33.89 $\pm$ 10.43*	< 0.05
A/G ratio	0.74 $\pm$ 0.19	0.51 $\pm$ 0.20*	0.39 $\pm$ 0.17*	0.38 $\pm$ 0.23*	< 0.05

Table 2

The results of total protein and absolute values of protein fraction (g/L) in normal dogs and dogs infected with *B.canis*, *E.canis* or *H.canis* single infections (mean $\pm$ SD). *P*-value – significance of the analysis of variance; n.s.: not significant. *Significant difference between normal and *B. canis*, *E. canis* or *H. canis* at *P* < 0.05.

Variables (g/L)	Normal (n = 9)	<i>B. canis</i> (n = 13)	<i>E. canis</i> (n = 20)	<i>H. canis</i> (n = 14)	<i>P</i> -value
Total protein	88.47 $\pm$ 7.26	68.25 $\pm$ 16.46	86.19 $\pm$ 22.26*	96.67 $\pm$ 30.65*	< 0.05
Albumin	24.22 $\pm$ 9.72	25.08 $\pm$ 8.34	22.85 $\pm$ 6.63	22.29 $\pm$ 4.70	n.s.
α 1-globulin	3.78 $\pm$ 2.44	4.08 $\pm$ 1.80	4.40 $\pm$ 2.04	3.57 $\pm$ 1.83	n.s.
α 2-globulin	4.00 $\pm$ 3.84	6.31 $\pm$ 4.71	9.40 $\pm$ 5.88*	7.00 $\pm$ 6.85	< 0.05
β 1-globulin	6.33 $\pm$ 4.66	8.00 $\pm$ 4.14	8.65 $\pm$ 4.99	10.79 $\pm$ 8.27	n.s.
β 2-globulin	12.00 $\pm$ 3.61	13.43 $\pm$ 12.14*	17.90 $\pm$ 8.44*	21.64 $\pm$ 13.60*	< 0.05
γ -globulin	13.67 $\pm$ 5.32	18.62 $\pm$ 13.55	23.00 $\pm$ 12.17*	34.86 $\pm$ 17.58*	< 0.05

The CRP concentration was detected by fluorescent immunoassay. The CRP concentrations in dogs infected with *B.canis* (n = 6) were between 52.0 and 200.0 mg/L, with an average of 127.62 mg/L; in dogs infected with *E.canis* (n = 6) they were between 50.2 and 166.7 mg/L, with an average of 105.35 mg/L;

and in dogs infected with *H.canis* (n = 6) they were between 10.0 and 51.2 mg/L, with an average of 45.6 mg/L.

Discussion

SPEPs is one of the standard tests used to monitor health and disease status such as infection and the acute and chronic inflammatory response. In this study of canine ehrlichiosis, babesiosis and hepatozoonosis, the average relative concentration of serum protein fraction albumin level and the A/G ratio were significantly lower than normal, whereas the absolute total protein of *H.canis* was significantly higher. A decreased albumin concentration is usually caused by liver damage, starvation or cachexia, digestive disorders, or kidney disease [12]. Hypoalbuminemia may result from loss of albumin through the kidney and/or the lack of synthesis of albumin in the liver. However, the pathogenesis of *B. canis*, *H. canis* and *E. canis* infection is usually caused by liver and/or kidney damage [9, 13]. The decreased A/G ratio resulted from a reduction in albumin concentration and an increase in globulin concentration. In previous reports, *E. canis*-infected dogs were found to have significant hypoalbuminaemia, hyperglobulinaemia and hypergammaglobulinaemia [14]. The SPEPs of *B. canis*-infected dogs showed decreased albumin concentration and A/G ratio but increases in α - and β -globulin concentrations. These abnormalities are due to haemolysis, hypertransferrinaemia due to dehydration, and anaemia [15, 16]. The total protein of *H. canis* was increased, probably due to hyperglobulinaemia caused by the stimulation of the humoral response of the organism to severe chronic inflammation [17, 18].

In the globulin fraction, the relative concentration and absolute values of β 2-globulin protein levels were significantly increased in *B. canis*, *H. canis* and *E. canis* infections. An increased β 2-globulin level may result from increased C3a (complement) protein concentration. Complements are involved in the regulation of inflammatory processes, and this complement protein plays a role in the development of intravascular hemolysis, especially in babesiosis [15, 16]. The γ -globulin fractions were increased in all three groups, with significantly increased absolute protein values in *E. canis* and *H. canis* infections. The γ -globulin fraction consists of different classes of immunoglobulins, and an increase in the fraction could lead to monoclonal gammopathy (narrow peak) or polyclonal gammopathy (broad peak). Monoclonal gammopathies result from a single line of B-lymphocytes or plasma cells, while polyclonal gammopathies are usually an indication of chronic inflammation and chronic liver damage [12]. In this study, we found monoclonal gammopathy in 35% of *E. canis* and 39% of *B. canis* single infections. In a previous study, polyclonal and benign monoclonal gammopathies were observed in *E. canis*-infected dogs [19]. In dogs with *H. canis* single infection, the serum protein pattern showed β - γ bridging and an increase in the β - and γ -globulin peaks in 43% of cases. The β - γ bridging pattern is usually found in liver disease [20]. The γ -globulin concentration of *H. canis* single infection differed significantly from that of other blood parasite infections because hepatozoonosis has a longer life cycle than others and also produces a chronic immune response [13].

CRP is a major acute-phase protein in dogs that is mostly synthesised in the liver following tissue damage caused by infection, inflammation or trauma. The acute-phase response is thought to be an

innate host defence mechanism occurring during tissue injury or immunological disorders and in the early stage of blood parasite infection. It is responsible for the accumulation and activation of granulocytes and mononuclear cells, which in turn release acute-phase cytokines, including interleukin-1 (IL-1), IL-16 and tumour necrosis factor alpha (TNF- α) [21]. In the current study, the average CRP concentrations in dogs with *E. canis*, *B. canis* and *H. canis* single infections were higher than the reference range (> 30 mg/L), and the average CRP concentration in *B. canis* single infection was higher than that of *E. canis* and *H. canis* single infections. In the case of canine hepatozoonosis, most dogs were within the normal range and a few were higher than the normal range. In a previous study, the CRP concentration in dogs infected with *E. canis* was shown to increase during the acute stage of infection; this increase might help to kill *E. canis* in the macrophages of infected dogs. The CRP level might help when assessing the severity of inflammatory damage in *E. canis*-infected dogs, and veterinarians may use this information to decide whether to use pro-inflammatory therapy [22]. The typical haematological abnormality of blood parasite infections, including ehrlichiosis, babesiosis and hepatozoonosis, is anaemia and/or immune-mediated anaemia (IMHA). Ehrlichiosis and babesiosis cause canine secondary IMHA [23, 24]. The serum CRP concentrations in canine autoimmune hemolytic anaemia and primary IMHA are increased [25, 26].

Conclusions

SPEPs in a blood parasite single infection mostly showed decreased albumin level and A/G ratio and increased β 2- and γ -globulin levels. The CRP concentration was usually increased in dogs with a single infection. SPEPs and the CRP level can help veterinarians monitor health status and blood parasite problems in sick dogs during the treatment process.

Materials And Methods

Sample collection

Six-hundred-and-fifty canine blood samples were collected from small animal hospitals and clinics in Bangkok and its vicinity. Samples were collected in EDTA tubes and serum collection tubes, and suspected blood parasite infections were examined by buffy coat thin blood smear and confirmed by PCR [8]. The numbers of normal dogs and those with *B. canis*, *E. canis* or *H. canis* single infections were 9, 13, 20 and 14, respectively. The criteria for normality were healthy dogs with no clinical signs of blood parasite infection and no history of ectoparasite infestation. All blood samples were negative for blood parasites, confirmed by PCR. Blood chemistry profiles were in the normal range [27]. All serum samples were kept at -20°C until analysis.

Serum protein profile determination and electrophoresis

Total serum protein was measured by biuret colorimetric test (Human[®], Wiesbaden, Germany). The positive serum protein samples were separated by agarose gel electrophoresis (SPIFE[®] split beta SPE kit,

Helena Laboratories, TX, USA). Twenty microlitres of serum sample was placed in each well and electrophoresis performed at 400 V for 6 min. The gel was pre-dried at 53 °C for 12 min, stained with acid blue staining solution and destained in citric acid destaining solution. All steps were carried out in an automated machine (Spife®3000, Helena Laboratories). The density of each serum protein band in the electrophoresis pattern was measured and analysed using QuickScan Touch software (Helena Laboratories).

Laboratory measurements of C-reactive protein concentration

The CRP concentrations of blood-parasite-positive serum samples were measured by fluorescent immunoassay (Vcheck Canine CRP 2.0 test kit, Bionote, South Korea). Five microlitres of each sample was diluted in diluent buffer from the test kit. One hundred microlitres of diluted sample was mixed and added to the test device and the CRP concentration displayed on the screen after 5 min. A CRP concentration above 30 mg/L was considered abnormal.

Statistical Analysis

The SPEPs data for blood parasite single infection were tested using ANOVA. The Tukey test was used for pairwise comparisons between single infections and normal dogs. Significance was set at $P < 0.05$.

Abbreviations

CRP
C-reactive protein
EDTA
ethylenediaminetetraacetic acid
SPEPs
serum protein electrophoresis patterns

Declarations

Acknowledgements

The authors would like to thank Assist. Prof. Chatree Khatiworavage for the statistical analysis. Authors would like to thank all Vet Central Lab staffs who contributed the retrospective data.

Authors' contributions

SA and PT: designed the study, correcting data, data analysis and interpretation, and draft the manuscript.

Funding

The Special Task Force for Activating Research, Chulalongkorn University (STF 6401531001-1).

Availability of data and materials

The datasets used and/ or analyzed in this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The research protocol was approved by institutional review board of Chulalongkorn University Animal Committee (approval no. 1931052). Informed consent was obtained from animal owners for using animal samples for the study. All methods were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Competing interests

There is no conflict of interest.

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Figures

Figure 1

Representations of the serum electrophoresis patterns of normal dogs (A) and dogs with single infection with *E. canis* (B), *B. canis* (C) and *H. canis* (D).