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CLINICAL AND HAEMATOBIOCHEMICAL STUDIES ON EXPERIMENTALLY INDUCED CHRONIC BABESIOSIS IN SPLENECTOMIZED ADULT DOGS

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ABSTRACT

The pathogenicity of the carrier *Babesia gibsoni* organism was studied in the experimentally inoculated carrier blood into the three susceptible splenectomized adult dogs during the period between 2000 to 2002. The pathogenic effect was assessed on clinical pictures and haematobiochemical changes. All the three experimentally infected dogs showed signs of depression, anorexia and anaemia (pale visible mucous membrane) at 2 years of post-infection. Haematological effects included macrocytic normochromic anemia with polychromasia, anisocytosis and a marked increased in nucleated erythrocytes. Biochemical changes included significant ($p < 0.05$) increase in mean total serum protein (8.27 ± 2.37), serum bilirubin (1.02 ± 0.11), total serum iron (279 ± 142.03) in comparison to control healthy dogs. It may be concluded from this study that the chronic canine babesiosis caused by *B. gibsoni* is highly pathogenic associated with anaemia and haematobiochemical alteration.

Key words: Haematobiochemical changes, chronic babesiosis, experimentally induced, splenectomized dog

INTRODUCTION

Canine babesiosis caused by large *Babesia canis* which is the most widespread and pathogenic, whereas, the small form, *B. gibsoni* produces a more chronic disease. The *B. gibsoni* infection was first identified by Patton (1910) in hounds and jackals in India and subsequently it has been reported in dogs from Ceylon (Seneviratna,1953) and Malaysia (Groves and Yap,1968), in foxes from Egypt (Maronopot and Guindy, 1970) and from a wolf in Turkestan (Yakimoff and Schokhor, 1917). The acute canine babesiosis characterized by pyrexia with remissions and exacerbations, anaemia and lassitude. Patients that survive become carriers, a state in which a harmless, subclinical infection is maintained by a delicate immunological balance between protozoa and antibodies. This balance is readily disturbed by the stress of transport, depression of food, pregnancy or intercurrent disease. However, the pathogenic and epidemiologic significance of carrier *B. gibsoni* organism are poorly understood. This paper describes the pathogenic and haematobiochemical effects of carrier *B. gibsoni* organism in experimentally induced splenectomized dogs.

MATERIALS AND METHODS

Three splenectomized adult dogs beagle of breed were inoculated with *B. gibsoni*-infected blood collected from others carrier dogs with 0 to 0.03% parasitemia, and used as *B. gibsoni*-infected dogs and clinically healthy six dogs which served as non-infected controls. Dog-1: two years before, Dog-2: three years before and Dog-3: five years before were inoculated with *B. gibsoni.* Three weeks after splenectomization 20 ml of infected blood was inoculated per of kg body weight. During the experimental period, parasitemia of the *B. gibsoni*-infected dogs varied from 0 to 0.30% in chronic stage, and haematocrit value from 14.6 to 41.4%. Clinical observations, peripheral blood smear and microscopic examinations were made in accordance with standard techniques. Blood samples were collected from *B. gibsoni* infected dogs using EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. Complete blood count was measured using an automatic cell counter (Celltac Nihon Kohden, Tokyo, Japan). Percentage of parasitemia was determined on a Giemsa-stained smear by counting the number of parasitized cells per 2000 cells. Reticulocytes were detected by microscopic examination of a blood smear stained with new methylene blue staining solution (Jain, 1986).

The staining solution contained 0.5% new methylene blue, 0.85% sodium chloride and 1% formalin. The reticulocytes were classified into an aggregate-type containing strings and clumps of bluish reticulum, and a punctate-type containing 4 or more separated dots of bluish material. Serum was prepared by allowing whole blood to clot by incubation at 37°C for 1 h followed by centrifugation at 514 x *g* for 15 minutes. The collected serum was filtrated by using milipore filter to remove any remaining blood cells. The biochemical tests such as total serum protein and total serum bilirubin from dogs infected with *B. gibsoni* were determined by using Automatic Diagnostic Analyzer (COBAS MIRA, Tokyo, Japan), and total serum iron and total iron binding capacity (TIBC/CPBA) were determined by the help of Biomedical Laboratories (BML, Sapporo, Japan). Statistical analysis was performed using Student's *t*-test.

RESULTS AND DISCUSSION

The clinical syndromes produced by *Babesia gibsoni* were observed and characterized by depression, anorexia and severe anemia inspite of very low parasitemia. The chronic form of the disease is poorly characterized, and infected dogs may become chronic carriers with important clinical signs (Table 1). In addition to these symptoms, infected Dog-3 showed very much severe long time carrier of infection.

Table 1. Clinical signs of chronically infected with *Babesia gibsoni* in adult dogs

S/N	Parameters	Non-infected control	Chronically infected		
			Dog-1	Dog-2	Dog-3
1.	Depression	-	+	+	++
2.	Anorexia	-	-	-	±
3.	Pale mucous membrane	-	±	+	++
4.	Fever (> 102°F)	-	-	±	±
5.	Emesis	-	±	+	+
6.	Dark brown urine	-	-	-	+
7.	Icterus	-	-	+	++
8.	Emaciation	-	-	-	+
9.	Rapid, theady pulse	-	-	-	±
10.	Diarrhoea	-	-	-	+
11.	Ataxia	-	-	±	+
12.	Edema	-	-	±	+

- = No symptoms, ± = Some times occurred, + = Mild symptoms, ++ = Severe symptoms.

The haemotologic findings of chronic babesiosis are presented in Table 2. Anaemia was the consistent sign of *B. gibsoni* infection. Parasitic relapses may occur with concomitant drops in the erythrocytes count. The blood picture was typically one of a macrocytic anaemia. There is a very active haematopoietic response, regenerative, as characterized by polychromasia, reticulocytosis and occasionally increased numbers of nucleated erythrocytes in the infection (Botros *et al.*, 1975 and Irizarry-Rovira *et al.*, 2001). Total leukocyte count (TLC) fluctuated resulting slightly leukocytosis. It also showed the biochemical changes in serum of dogs infected with *B. gibsoni*. Parameters of serum such as total protein, bilirubin, iron and iron binding capacity were varied in serum of infected dogs.

Although these values were not tested statistically but these values varied widely in different chronic stages of infection. The total serum protein (TSP) concentrations in chronic canine babesiosis varied widely from 6.8 to 11 μg / dl with an average of 8.27 ± 2.37 μg / dl which was significantly ($p < 0.05$) differ from healthy dogs (6.35 ± 0.07) (Table 2).

Table 2. Certain hematobiochemical changes of experimentally induced chronic canine babesiosis in adult splenectomized dogs

S/N	Parameters	Unit	Healthy control	Chronically infected			Mean ± SD
				Dog-1	Dog-2	Dog-3	
1.	Haematocrit	%	49.95 ± 0.49	41.40	36.90	14.60	30.97±14.35*
2.	Haemoglobin	g/dl	15.80 ± 0.16	13.50	11.50	05.10	10.03±04.39
3.	Erythrocytes	10^6/mm^3	08.90 ± 0.61	05.85	03.85	02.11	03.94±01.88*
4.	MCV	fl	64.50 ± 5.57	68.00	71.00	69.00	69.33±01.52
5.	MCH	pg	21.85 ± 1.67	21.50	23.10	24.20	22.22±00.80
6.	MCHC	g / dl	33.80 ± 0.91	31.40	32.60	34.90	32.97±01.78
7.	Leukocytes	10^3	06.15 ± 0.91	07.30	08.50	10.20	08.67±01.46
8.	Platelets	10^3	32.30 ± 5.57	17.40	16.30	02.50	12.07±08.30
9.	Total reticulocytes	%	00.15 ± 0.07	01.80	06.50	10.70	06.33±04.45*
10.	Puncutate type	%	00.05 ± 0.02	00.20	02.10	00.50	00.93±01.02*
11.	Aggregative type	%	00.10 ± 0.05	01.60	04.40	10.20	05.40±04.39*
12.	Anisocytosis	-	-	+	+	+	-
13.	Polychromasia	-	-	-	±	+	-
14.	Howell Jolly bodies	-	-	-	-	±	-
15.	Total serum protein	g / dl	06.35 ± 0.07	07.00	06.80	11.00	08.27±02.37*
16.	Total bilirubin	μg / dl	00.10 ± 0.00	01.07	00.90	01.10	01.02±00.11*
17.	Unconjugated	μg / dl	0	00.30	00.25	00.40	00.32±00.08
18.	Conjugated	μg / dl	00.10 ± 0.00	00.77	00.65	00.70	00.71±00.06
19.	Total serum iron	μg / dl	198.50 ± 0.7	196.0	198.0	443.0*	279.0±142.03*
20.	Total iron binding capacity (TIBC / CPBA)	μg / dl	386.50±19.1	400.0	333.0	411.0*	381.33±42.2

– = not present, ± = some times present, + = always present, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration. *Indicates significant at ($p < 0.05$).

Average bilirubin value in healthy control dogs was 0.10 g /dl whereas in experimentally induced *B. gibsoni* infection, there was an increase in total bilirubin up to 10 times in chronic stages which was significantly ($p < 0.05$) differ. Fowler *et al.* (1972) reported an increased total bilirubin up to 4.3 μg / dl. The most of the increased bilirubin was conjugated type. Total serum iron and iron binding capacity were increased significantly ($p < 0.05$) in very chronic infected Dog-3. Although chronically infected Dog - 1 and Dog – 2 were differ in this observation.

The mean haematocrit value, erythrocyte count, total reticulocytes in chronically infected dog were 30.97 ± 14.35, 3.94 ± 1.88 and 6.33 ± 4.45 respectively. These values were significantly ($p < 0.05$) lower in comparison to healthy dogs. In each case, the diagnosis of babesiosis was confirmed by finding the parasites in Giemsa-stained blood smears. We could not find parasites in the blood of three dogs at a time of clinical chronic stage of illness but were seen on subsequent examination. Finding of *B. gibsoni* organisms required good staining and considerable practice. Many of the erythrocytes in these anemic dogs were vacuolated and pitted, making recognition difficult. The parasites were especially difficult to find using Giemsa-stained smear but sometimes examination of 2 or 3 slides to find a parasitized cell was required. It is our hope that the idea presented in this paper will stimulate to help the diagnosis of chronic canine babesiosis for the small animals veterinary practitioners.

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