

Babesia Canis and Ehrlichia Canis: Cause of Reactive Hemophagocytic Syndrome

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Abstract: Dogs can be coinfecting with *Ehrlichia* and *Babesia* species. The objective of the present study is to report a fatal case of non-experimental babesiosis and ehrlichiosis in a 1 year old female Rottweiler, pregnant, with prominent HFS (haemophagocytic syndrome) and intense parasitemia. To our knowledge, we describe a rare case of this peculiar association (HFS) in a dog with babesiosis and ehrlichiosis. This case was diagnosed in a laboratory located in Galicia, northwest Spain. The dog had serological evidence of ehrlichiosis with typical *E. canis* morulae and *Babesia canis* piroplasm detectable in blood smears. Hemophagocytic macrophages was reported in blood smears and bone marrow aspiration smears. The main characteristics of the syndrome were an intense parasitemia and regenerative anaemia, thrombocytopenia, leucopenia, hepatic affection and evolution towards renal failure.

Key words: Tick-borne infection, *Babesia canis*, *Ehrlichia canis*, Canine infection, Hemophagocytic syndrome, Pregnancy and Bone marrow

Introduction

Babesiosis is a tick-borne malarialike illness caused by species of the intraerythrocytic protozoan *Babesia*. Two out of the 73 identified *Babesia* species are known to cause natural infection in dogs in Galicia, Spain: *Babesia canis* (Camacho *et al.*, 2003) and *Babesia microti* (Camacho *et al.*, 2001). Of both, *Babesia canis* is considered to be the most important specie affecting dogs. It has a relatively large size (2.4 μm x 5.0 μm), piriform shape and can appear in red blood cells singly or in groups of 2, 4 or even more (Greene, 1998). The pathogenicity of the parasite is highly determined by the species involved and the immunologic response of the host. Among other immunologic mechanisms, infected erythrocytes incorporate *Babesia* antigens into their membranes inducing antibodies that opsonize the red cells leading to their removal by the mononuclear-phagocytic system (Tizard, 1996).

The etiologic agent of canine ehrlichiosis, the rickettsia *Ehrlichia canis* (*E. canis*), parasitizes circulating monocytes intracytoplasmically in clusters of organism called morulae. The disease is

acknowledged as an important and potentially fatal infectious disease of dogs and other members of de *Canidae* family. During the acute phase, the parasite enters the blood stream and lymphatics and localizes in macrophages of the reticuloendothelial system in the spleen, liver and lymph nodes, where it replicates by binary fission. Diagnosis of ehrlichiosis is confirmed by visualization of the morulae in circulating monocytes, detection of increased serum antibodies to *E. canis*, or by the demonstration of *E. canis* DNA by polymerase chain reaction (PCR). The presence of hemophagocytic macrophages in canine bone marrow has been associated with a variety of disorders. In general, these can be divided into 2 major clinical syndromes: malignant histiocytosis and reactive hemophagocytic syndrome.

The reactive haemophagocytic syndrome (HFS) is a disorder characterized by a proliferation of non-neoplastic mature macrophages in liver, spleen, lymph nodes, lungs and bone marrow or some combination of these sites, with prominent haemophagocytic activity, associated with

peripheral pancytopenia (Auerbach *et al.*, 1986). This peculiar association is a pathologic finding in the context of several infectious diseases (viral, bacterial, parasitic or fungal infections) (Aouba *et al.*, 2000).

The objective of the present study is to report a fatal case of non-experimental babesiosis and ehrlichiosis in a 1 year old female Rottweiler, pregnant, with prominent HFS and intense parasitemia.

Materials and Methods

In January 2002 a 1 year old female, Rowttweiler, pregnant, was admitted to a veterinary hospital presenting hyperthermia (40°C), trembling, back paraparesis, dark urine and vomits, of several days of evolution. The dog lived in a rural area of Galicia, north-west Spain. Physical examination revealed pale mucous membranes with petechiae, mouth sores, tachycardia, tachypnoea, hepatosplenomegaly, lymphadenopathy and the presence of ticks (1 male and 2 loaded females of *Rhipicephalus sanguineus* and 2 females without feeding of *Ixodes hexagonus*).

Blood sample was obtained by puncture in the cephalic vein during the acute febrile syndrome. Haematological count and different serum biochemistry tests were carried out by a STKS-2 VCS automatic counter (Coulter®) and a clinical chemistry analyser CL-7200 (Shimadzu®). Serum protein electrophoresis was conducted by a Paragon CZE® 2000 (Beckman Coulter®). Microscopic examination of brilliant cresyl blue and Giemsa-stained peripheral blood smears were performed. Both staining techniques were performed as described by Henry, 1996. The study of the presence of anti-*E. canis* antibody was made with the immunofluorescent antibody (IFA, VMRD, Inc., USA). Despite treatment with imidocarb dipropionate (Imizol®, 4 mg/Kg, SC), doxyciclin (Vibracina®, 60 mg/Kg/12 hours, PO) and methylprednisolon-6 α (Urbason®, 25 mg/Kg/8 hours, IV) the dog was euthanatized within 1 week after the onset of the symptoms because of their deteriorating clinical condition. Bone marrow aspiration smears was obtained at the time to euthanasia.

Results and Discussion

Based on the visual observation of the parasite *Babesia canis* in Giemsa-stained blood smears and visualization of the morulae in circulating monocytes, a diagnose of babesiosis and ehrlichiosis was made for the acute febrile syndrome. The main characteristics of the

syndrome were an intense parasitemia and regenerative anaemia, thrombocytopenia, leucopenia, hepatic affectation and evolution towards renal failure.

Results of the haematological count confirmed the clinical impression of a marked anemia (Table 1). The anemia would have clear regenerative characteristics as reticulocyte count and presence of nucleated red cells demonstrated. The high values obtained for the red cell distribution width (RDW) support this finding, since anisocytosis would reflect the presence of two erythrocytic populations: a normocytic one represented by erythrocytes and a macrocytic one corresponding to reticulocytes. Behaviour of the levels of LDH (lactate dehydrogenase) and BT (total bilirubin) are also in agreement with the intense haemolytic regenerative anemia.

During the acute febrile syndrome an important thrombocytopenia and leucopenia was observed. The presence of giant platelets was visually detected in blood smears and is consistent with the regenerative character of the thrombocytopenia. Leucopenia has been frequently reported by other authors in relation with HFS (Auerbach *et al.*, 1986). Differential white cell count clearly showed a tendency towards neutropenia and an increase in the number of monocytes and lymphocytes.

Examination of giemsa-stained peripheral blood smears showed abundant erythrocytes parasitized cells with *Babesia canis* parasites (22%), with a pyriform structure (Fig. 1), intra and extracellulars and revealed marked haemophagocytic activity. These cells were large in size and had atypical features including anisocytosis, round or oval nuclei, fine nuclear chromatin and prominent and/or multiple nucleoli. The erythrofagocytosis could be related to the activation of the macrophage-monocyte system as a consequence of the disease, a previously reported finding by Taboada and Merchant, 1991. The cells phagocytized by macrophages are most likely the result of opsonization of erythroid cells as a result of antibody binding. Phagocytic macrophages presumably are an appropriate response to the need to remove cellular debris (Weiss *et al.*, 1985). Other findings were the presence of atypical lymphocytes and vacuolated macrophages (many of these cells contained large numbers of cytoplasmic vacuoles). Bone marrow aspiration smears had 3% hemophagocytic macrophages. Macrophages were large (>25 µm) and resembled reticulum cells (reticular-type cells), with lacy chromatin and 1 to 3 nucleoli. Many of these cells contained

Table 1: Results of haematological and serum biochemistry analysis of a female Rowtweiler with *Babesia canis piroplasm* and *Ehrlichia canis*

Parameter (normal reference range and units)	Acute phase
Leucocyte count (6.0-17.0 x 10 ⁹ cells/litre)	4.9
Neutrophils (60-77%)	33.6
Lymphocytes (12-30%)	38.6
Monocytes (3-10%)	27.1
Eosinophils (2-10%)	0.5
Basophils (0-1%)	0.2
Red blood cell count (5.5-8.5 x 10 ¹² cells/litre)	2.57
Reticulocytes (0.5-1-5 per cent)	3.9
Haemoglobin (120-180 g/litre)	64
Haematocrit (37.0-55.0 per cent)	18.7
Red cell distribution width (<19.0 per cent)	22.3
Platelet count (120-500 x 10 ⁹ cells/litre)	41
Glucose (3.44-6.0 mmol/litre)	3.05
Urea (3.16-9.16 mmol/litre)	47.4
Creatinine (44.2-141.4 µmol/litre)	468
Aspartate aminotransferase (8-57 U/litre)	687
Alanine aminotransferase (8-48 U/litre)	934
γ- Glutamyltransferase (5-16 U/litre)	8
Alkaline phosphatase (10-120 U/litre)	302
Total bilirubin (1.71-10.26 µmol/litre)	129
Triglycerides (0.11-1.32 nmol/litre)	1.92
Cholesterol (3.02-6.6 mmol/litre)	8.90
Lactate dehydrogenase (65-150 U/litre)	631
Total proteins (55-75 g/litre)	79
Albumin (23-34 g/litre)	20
α1-globulin (2.0-5.0 g/litre)	4.8
α2-globulin (3.0-11.0 g/litre)	8.6
β-globulin (1.0-27.0 g/litre)	23.6
γ-globulin (5.0-12.0 g/litre)	22.0

large numbers of cytoplasmic vacuoles. Diagnosis of ehrlichiosis is confirmed by visualization of the morulae in circulating leucocytes and detection of increased serum antibodies to *E. canis*. The presence of anti-*E. canis* antibody titers (IFA) at a dilution 1/1280 is considered confirmation of infection. The indirect immunofluorescent antibody (IFA) test using *E. canis* antigen is the most acceptable serological test. In the acute stage of the disease when dogs are clinically ill, antibody titers increase rapidly. The role of babesiosis as a risk factor for renal failure had been previously proposed (Lobetti et al., 1996). Mechanisms of acute nephropathy are discussed (immune-complex formation and/or tubular necrosis due to hemoglobinuria). In the case we present, signs of an acute renal failure were observed, with urea and creatinine levels highly elevated, together with hypercholesterolemia, hypertriglyceridemia and hypoalbuminemia. Hepatic affectation have been

reported in babesiosis and ehrlichiosis (Pagès et al., 1990). In this case, elevation of serum AST (aspartate aminotransferase) and ALT (alanine aminotransferase) values was very high and could be mainly associated with direct hepatic damage. The hypoglycaemia present has been shown to be correlated to increased plasma TNF (tumour necrosis factor) levels (Clark et al., 1992). Our patient had the 6 clinical criteria for the diagnosis of HFS (fever, anaemia, thrombocytopenia and/or leucopenia, hypertriglyceridaemia, hepatosplenomegaly and lymphadenopathy) and according to these criteria the diagnosis of HFS is established (Favara, 1992). The spectrum of babesiosis and ehrlichiosis is broad and ranges from asymptomatic carrier states to fulminant disease that results in rapid death. The importance of both in companion animals is well-recognized in parts of the world where virulent strains exist and where the

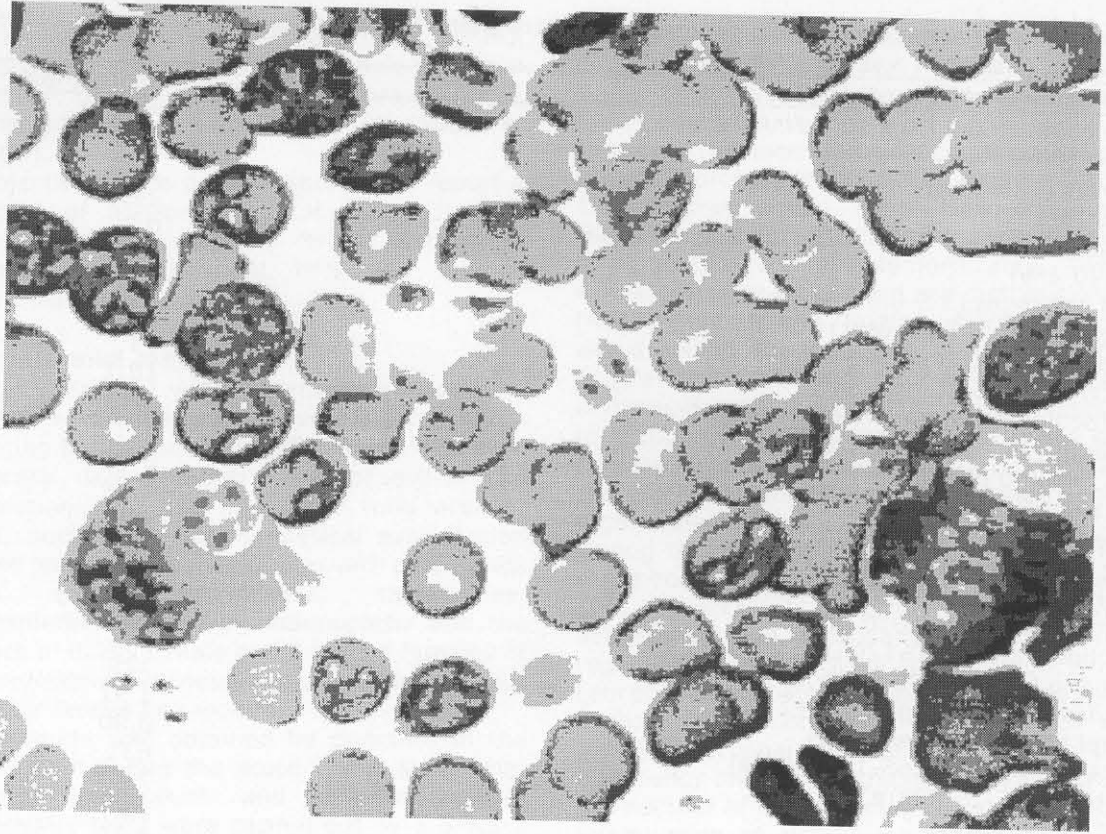


Fig. 1: Giemsa-stained peripheral blood smear from a dog with *Babesia canis* and *Ehrlichia canis*. Ehrlichia morulae in circulating monocytes

diagnosis and treatment of both typical and atypical presentations is part of the everyday course of events for the small animal clinician.

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