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CLINICAL STUDY AND PATHOLOGICAL FINDINGS ON BABESIOSIS IN DOGS, ON SEASIDE OF ROMANIA

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Abstract

Canine babesiosis is a protozoan tick-borne disease affecting dogs worldwide. Knowledge on the prevalence and clinico-pathological aspects of Babesia species infecting dogs is of epidemiological and veterinary medical interest. Here we describe twenty cases of clinical babesiosis diagnosed in dogs, between March-June 2016, in a Veterinary Clinic located in the seaside (Dobrogea region) of Romania. Dogs with clinical signs compatible for babesiosis and positive when tested for the presence of intraerytrocytic protozoan parasites using the blood smear method were included in the study. Of the total animals, the most affected were adults (over 8 years of age); dogs of Bichon Maltese breed and male animals appear in higher numbers. The clinical presentation, pathological findings and therapeutic approaches are discussed in three clinical groups: mild, moderate, and severe babesiosis. Group one comprises dogs (n=9) with mild babesiosis characterized by lethargy, anorexia, fever without major changes in the hematological parameters; no other organ pathology. Dogs (n=5) with moderate babesiosis showed at least one change in the hematological (anaemia, thrombocytopenia, lymphopenia, neutropenia/neutrophilia, leukocytosis) and/or biochemical (elevated levels of liver enzymes, blood urea nitrogen, and creatinine, low albuminemia) parameters, reflecting an organ disorder. The third group included dogs (n=6) which developed complications associated with babesiosis, presenting at least two of the following complications: acute renal failure (n=3), hepatopathy (n=4), pancreatitis (n=2), acute respiratory distress syndrome (n=1). All animals were treated with imidocarb. Additionally, for dogs with moderate and severe babesiosis, a symptomatic treatment (intravenous fluid therapy, antiinflamatory, antypiretic, antiemetic, antispastic, procoagulant drugs) was administrated, while four dogs with severe anemia (PCV: 14 -27.57%) needed blood transfusion, too. The recovery rate (100%, 100%, and 50%, respectively) for the three clinical groups and mortality rate (0, 0, and 50%, respectively) revealed that a successful treatment is depending on the severity of diseases and the individual response of the host.

Key words: *clinical signs, pathology, therapy, babesiosis, dogs.*

INTRODUCTION

Babesiosis is an important tick-borne zoonotic diseases caused by intraerytrocytic protozoan species of the genus *Babesia* (Berger and Marr 2006; Uilenberg, 1995). These parasites usually affect vertebrate animals and are transmitted by various species of ticks. In dogs, infection with these hemoparasites leads to a wide range of clinical signs, of subclinical disease up to serious illnesses characterized by fever, jaundice, splenomegaly, weakness and collapse associated with intra- and extravascular hemolysis, hypoxia, systemic inflammatory response, thrombocytopenia and haemoglobinuria (Jacobson, 2006).

Traditionally, babesiosis in dogs is diagnosed based on the morphology of the intraerythrocytic piroplasm merozoites observed by microscopic examination in peripheral blood smears. By this method, the piroplasms can be classified as large (e.g. *Babesia canis*,) or small forms (*Babesia gibsoni*). However, currently, the large piroplasm forms previously considered to be *B. canis*, include *B. canis*, *Babesia rossi* and *Babesia vogeli* as distinct species (Carret et al. 1999).

Despite of their identical morphology, although they have significant differences in their clinical presentation, geographical distribution and vector specificity (Uilenberg, 1996).

Clinically, canine babesiosis can evolve in various forms from subclinical to super-acute. The severity of clinical signs and therefore the lesion framework depends on several factors such as species of the causative agent, immune status of the host and the existence of inter-

current illness (Irwin, 2009; Solano-Gallego and Baneth, 2011).
For example *B. rossi*, the dominant species in South Africa, is very virulent, with highly acute clinical evolution.
It is believed that the clinical signs are due to tissue hypoxia caused by anemia and systemic inflammatory response (Lobetti, 2006).
Anemia pathogenesis is not fully understood, intra- and extravascular hemolysis could be a cause, on the one hand and weak bone marrow response on the other hand. Mortality in the case of infection with *B. rossi* reaches up to 12% and 1% in the case of infection with *B. vögeli* (Lobetti, 2006).
Cases of babesiosis in dogs have been reported in different parts of the world. Global incidence of clinical babesiosis in dog is of 0.7% with variations depending on the countries and regions (Lenaig et al., 2014).
The status of the endemic country for *B. canis* was assigned to countries such as France, Spain, Hungary, Scotland, Germany, Belgium, Netherlands (Jacobson, 2006); for endemic *B. vögeli* are considered: the USA, Japan, Australia, South Africa, Brazil and for *B. gibsoni*: the USA, North East of Africa, Asia, Australia, Hungary, Italy (Jacobson, 2006).
In Romania, canine babesiosis is registering an increased prevalence (Imre et al., 2010; Ionita et al., 2012), particularly in the areas where in the last several years an increase in the tick population has been reported (Ioniţă and Mitrea 2003; Ionita et al. 2010).
Knowledge on the prevalence and clinico-pathological aspects of *Babesia* species infecting dogs is of epidemiological and veterinary medical interest.
Therefore, the aim of this study is to describe the clinical presentation and laboratory abnormalities of canine babesiosis, assessing its implications in the pathology of other organs and in the effectiveness of the babesiicid treatment.

MATERIALS AND METHODS

Within the study, there are included twenty dogs diagnosed with babesiosis, during March-June 2016, in a veterinary clinic for pets, located in the Dobrogea area (Constanta city) (table 1).
Suspicion of babesiosis was established based on clinical examination, respectively animals that displayed the following symptoms: febrile syndrome, anaemic mucous, jaundice, lethargy, anorexia, vomiting, haemoglobinuria, petechiae on gingival mucosa.
To confirm the diagnosis, blood samples have been collected for haematological (blood counts, smear) and specific biochemical (glucose-Glu, amylase-AMY, Glutamate oxaloacetic transaminase-GOT, Glutamate pyruvate transaminase-GPT, creatinine-CRE, blood urea nitrogen -BUN, total-billirubin- T-BIL, Ca, lactatdehidrogenase-LDH, alkaline phosphatise-ALP, albumine-Alb) parameters.
Blood analysis was performed using the Abacus Vet Jr. haemo-analyzer.
Biochemical determinations were carried out with the SPOTCHEM EZ SP-4430 device.
Thin blood smears were prepared, stained using a Dia-quick Panoptic kit and subsequently examined by light microscopy at 1,000x for detection of intraerytrocytic piroplasms) (Ionita and Mitrea, 2013).
In addition to haematological and biochemical analysis, rapid tests were used to detect the presence of other haemoparasites (4Dx IDEXX SNAP Test) or check the exocrine pancreatic function (CPL IDEXX) or exclude the presence of viral diseases (CDV Test).
The final diagnosis of babesiosis was established by the correlation of clinical signs, haematological analysis and identification of intraerytrocytic forms of *Babesia* spp. (Ionita and Mitrea, 2013).
Consequently to laboratory tests and clinical evolution, animals diagnosed with babesiosis were divided into 3 groups: mild, medium, and severe form of babesiosis.
Animals have been treated with specific babesiicid drug, but also supporting medications, according to their clinical status.
Anamnesis, clinical observations and the subsequent evolution of the animals were recorded in the database of the hospital.

RESULTS AND DISCUSSIONS

The clinical study, carried out on a total number of 20 dogs of different breeds, aged between 1 and 10 years old. All the animals had an owner and they originated from the

urban area of Dobrogea region, respectively: Constanta (n=16), Tulcea (n=3) and Cogealac (n=1) (table 1).

Table 1. General data on the dogs diagnosed with clinical babesiosis, included for the clinical follow-up

Nr. crt.	Place of origin (city)	Breed	Age (years)	Gender	Clinical form of babesiosis
1.	Constanta	Maltese Bichon	8	F	mild
2.	Constanta	Maltese Bichon	8	M	moderate
3.	Constanta	Maltese Bichon	1	M	moderate
4.	Constanta	Maltese Bichon	3	F	moderate
5.	Constanta	Maltese Bichon	6	M	severe
6.	Constanta	Maltese Bichon	1	M	mild
7.	Constanta	Maltese Bichon	2	F	mild
8.	Constanta	Maltese Bichon	4	F	moderate
9.	Constanta	Golden Retriver	1	F	mild
10.	Constanta	Golden Retriver	2	M	mild
11.	Tulcea	Metis	8	F	severe
12.	Constanta	Labrador Retriever	9	F	mild
13.	Constanta	Beasle	7	M	mild
14.	Cogealac	Shepherd of Central Asia	2	F	severe
15.	Tulcea	Carpathian Shepherd	3	M	severe
16.	Constanta	Metis	2	M	moderate
17.	Constanta	Labrador Retriever	8	M	mild
18.	Constanta	Rottweiller	6	M	mild
19.	Tulcea	Metis	10	M	severe
20.	Constanta	Alaskan Malamute	1	M	severe

Consecutively to clinical examination and laboratory tests, animals diagnosed with babesiosis have been divided into 3 groups: group 1- mild, group 2 - moderate, and group 3- severe babesiosis.

In the group 1, consisting of 9 animals who displayed mild forms of babesiosis, were included those animals showing on clinical examination at least one of the following clinical signs: lethargy, anorexia, fever, but without major changes on the haematological and biochemical parameters.

Dogs in the second group, with moderate forms of babesiosis (n = 5), in addition to clinical signs described in the group 1, had pronounced abnormalities in the blood and biochemical parameters, respectively: thrombocytopenia, lymphopenia, neutropenia/neutrophils, leukocytosis; increasing glucose, pancreatic amylase, creatinine, glutamate pyruvate transaminase and aspartate aminotransferase, and decreased albumin (Table 2, 3).

The third group includes animals who have presented severe form of the disease characterized by complications associated with babesiosis: renal failure (n=3), liver disease (n=4), pancreatitis (n=2) and respiratory failure (n=1).

Table 2. Haematological abnormalities in dogs (number; %) with different clinical forms of babesiosis

Haematological abnormalitiies	Dogs (n=20)		
	(n)	(%)	ND
Neutropenia	4	20.0	0
Neutrophilia	4	20.0	0
Lymphocytopenia	13	68.4	1
Granulocytosis	4	20.0	0
Mild thrombocytopenia (PLT > 70)	2	10.0	0
Moderate thrombocytopenia (PLT < 70)	4	20.0	0
Severe thrombocytopenia (PLT = 0)	14	70.0	0
Non-regenerative anaemia	5	25.0	0
Regenerative anaemia	2	10.0	0

Most dogs have been diagnosed with babesiosis in the beginning of the warm season (March-April) which shows the correlation between the appearance and the increased activity of vectors that transmit the parasite and the number of cases recorded (Ionita et al., 2010).

The majority of cases was registered in March (n = 8) with a small decrease in April (n= 5) and May (n= 5), then a significant reduction in June (n = 2).

In terms of gender, we have find that males have held the majority of new cases diagnosed with a prevalence of 60% (n=12) whereas females accounted for 40% (n= 8) of the total cases diagnosed with babesiosis.

In terms of clinical forms of babesiosis manifested, the mild form was registered in 5 males (55%) and 4 females (45%), the moderate form in 3 males (60%) and 2 females (40%) and the severe form in 3 males (50%) and 3 females (50%).

As the age at which animals have been diagnosed with babesiosis, the occurrence average is of 4.5 years old; the highest incidence was registered in dogs of ≥8 years old- 30% (n = 6), the rest were animals with age varying from 1 to 7 years old.

In terms of breed, high rate of infection (40%) occurred in the Maltese Bichon breed (n = 8).

As clinical manifestations, the febrile syndrome was recorded in all 20 dogs on their first consultation. Clinical manifestation such as vomiting was seen in 5 animals (25%), haemoglobinuria was detected in 3 (05%), pale mucous membrane was recorded on 19 (95%) and jaundice in a single dog (5%).

From the haematological point of view, dogs diagnosed with babesiosis presented neutrophilya / neutropenia, granulocytopenia, lymphocytopenia, thrombocytopenia of different degrees, as well as in the moderate and severe cases we have ascertained the presence of non-regenerative anemia (Table 2). In some dogs (n=2), severe anemia (PCV: 14 - 27.57) was registered.

According to biochemical analysis, within the classified groups there could be medium and severe cases, we have recorded overall increases in hepatic transaminases levels (GOT / GPT), pancreatic amylase, creatinine, BUN, total bilirubin, alkaline phosphatase, lactate dehydrogenase and glucose (Table 3, 4).

Table 3. Biochemical changes recorded in dogs who presented a moderate form of babesiosis

Biochemical parameter	No. of dogs in which it was determined	High values (n)	Low values (n)
Glu (glucose)	3	1	0
ALP (alkaline phosphatase)	1	1	0
GPT (glutamate pyruvate transaminase)	4	3	0
CRE (creatinine)	4	2	0
Amy (amylase)	2	1	0
T-Bil (total bilirubin)	3	1	0
GOT (glutamate oxaloacetic transaminase	3	2	0
Alb (albumin)	2	0	2
LDH (lactat dehydrogenase)	1	1	0

Table 4. Biochemical changes recorded in dogs with a severe form of babesiosis

Biochemical parameter	No. of dogs in which it was determined	High values (n)
BUN (blood urea nitrogen)	5	3
ALP (alkaline phosphatase)	1	1
GPT (glutamate pyruvate transaminase)	4	4
CRE (creatinine)	6	3
AMY (amylase)	5	3
T-Bill (total bilirubin)	4	2
GOT(glutamate oxaloacetic transaminase)	6	2
Albumine	1	1

Treatment was based on two main objectives: stabilizing the patient and babesiicid medication administration at the right time in terms of clinical status of the patients. Treatments were performed generally in outpatient and only 4 dogs have been admitted, of which 2 have died.

All animals were treated with imidocarb (dose of 4.25 mg/kg). In addition, to dogs with mild and moderate forms of babesiosis, a symptomatic treatment was administered (intravenous fluids, anti-inflammatory and antipyretic medications, antispasmodics and procoagulant medications) (Table 5).

Table 5. Therapeutical approaches (medications) used for the symptomatic and etiologic treatment of babesiosis

Drug / Medication	Effect	Dosage and administration
NaCl 0,09%	Rehydration, correction of electrolyte balance	l0-20ml/kg iv
Glucose 5%	Energetic support	5ml/kg iv
Lactated Ringer	Acidosis, uremia	5ml/kg iv
Duphalyte	Support of amino acids, energetic substrate	5ml/kg iv
Sodium Metamizol	Anti-pyretic	50 mg/kg iv, im
Drotaverine hydrochloride	Anti-spasmodic	1-2 ml sc. Im at 12-24 hours
Metoclopramide	Anti-vomiting	0.2-0.5 mg/kg sc at 6-8 hours
Maropitant	Central anti- vomiting	1 mg/kg sc. once a day
Ranitidine	Antacid	2 mg/kg iv 8-12 hours
Vitamin B Complex	Support	1-2 ml sc./ im
Vitamin C	Antioxidant, vascular tonic	0,5-1 g iv
Epinephrine	Cardiac arrest resuscitation	0.01 -0.02 mg/kg initially and if an appropriate response is not obtained, was increased up to 0.1-0.2 mg/kg iv
Pancreatic enzymes	Digestion maintenance after an episode of pancreatitis	according to the prospectus
Sylimarina	Hepatoprotector	according to the prospectus
Imidocarb Dipropionate (imizol solution 12%)	Babesiicid	6.05 mg/kg boosted 14 days

Furthermore, dogs who have shown severe anemia (PCV: 14 - 27.57) blood transfusion was required. The process of blood transfusion was carried out in 4 dogs (20%) of the patients which showed a severe form of babesiosis. Besides these clinical findings such as pale mucous membranes / jaundice, tachycardia,

tachypnea, they have also showed decreasing values of RBC, HGB, HCT at the haematological examination (RBC<3.85 $x10^{12}$/1; HGB < 9.2 mg/dI; HCT < 27.57%).
The recovery rate was of 100% in dogs with a mild form and moderate babesiosis and 50% in dogs with severe babesiosis.
The mortality rate for dogs who have manifested mild and moderate babesiosis and 50% for patients with a serious form suggests that successful treatment depends on the severity of the disease and on the individual response of the host.

DISCUSSIONS

In Romania, dog babesiosis has become a quite common parasitic disease in recent years, with a variety of clinical signs ranging from mild to moderate signs, from nonspecific clinical signs to collapse and death (Ionita et al., 2011). The severity of clinical babesiosis is characterized by marked hemolytic anemia severe acid-base imbalances (Leisewitz et al., 2001) resulting in kidney failure, liver disease with jaundice, hypoglycemia (Keller et al., 2004), acute respiratory failure, cerebral pathology and immune-mediated hemolysis (Jacobson, 2006). The severity of clinical signs and therefore the lesion framework depends on several factors such as breed causative agent, host immune status and inter-existing diseases (Lobetti, 2006).
According to this study, in Dobrogea, babesiosis in dogs presents an increased incidence in the period of March-June months, with a different aspect than the incidence described in Hungary (Mathe et al., 2006) where the authors observed an increased incidence in the summer-autumn months.
The haematological framework as well as other clinical changes were similar to those described in the literature (Duh et al., 2004; Furanello et al., 2005; Gopequi et al., 2007).
Among complications, liver failure, renal failure and pancreatitis were the most common deviations that we have found in dogs infected with *Babesia*. Liver failure in most cases was mild thus it did not even affect the course of therapy and recovery. Jaundice occurred in a single patient was not caused by the suffering of hepatocytes but it was determined by hemolysis.
Renal failure occurred in 5 dogs; in two of them an increased pancreatic amylase was also observed. As far as pathology is concerned, kidney failure is a common complication in dogs with babesiosis. Its gravity is directly responsible for the clinical course and survival rate (Mathe et al., 2006). In the study carried out on the pathogenesis of canine babesiosis in Hungary, the authors have established a threshold limit of creatinine in terms of survival rate (CRE = 275 μmol/l). In our study, a dog had an increased creatinine of 12.4 mg / dl (1096.41 μmol/l) while at another it was of 10.3 mg/dl (910.73 μmol/l). In these two patients, the major importance for survival was given by age and by inter-current pathologies, thus the individual with higher levels of creatinine survived while the other not, which demonstrates the relationship between clinical babesiosis and inter-current pathologies.
As a therapeutic babesiicid option, we have used imidocarb dipropionate with very good results, as suggested by other studies (Mathe et al., 2006; Uilenberg et al., 1981, Solano Gallego, 2008).

CONCLUSIONS

According to this study, babesiosis can have different types of clinical evolution, depending on different factors.
Clinical manifestations were represented by fever, anorexia, anemia / jaundice, haemo-globinuria, prostrate condition, muscle weakness. As babesiosis involvement in the pathogenesis of other organs, we have noticed alterations in the liver, renal and pancreatic function.
Treatment success was largely depended on the pathological form of the disease, on the evolutionary type and presentation time of animals for investigations. Age and inter-current pathologies have played an important role in the evolutionary process of the disease.
We have achieved good therapeutic results using dipropionate imidocarb as babesiicid, associated with supportive symptomatic medication, depending on each case, with a high recovery success.

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