CORRELATIONS REGARDING THE DIAGNOSIS AND THE OPTIMAL THERAPEUTIC PROTOCOL IN CANINE BABESIOSIS

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Abstract

Canine babesiosis is one of the most important vector-borne diseases worldwide that affects dogs regardless of age, breed or gender. The aim of this study was to corroborate the clinical signs of canine patients confirmed with babesiosis, the results of paraclinical investigations, as well as the choice of the therapeutic protocol. The present study was performed on 42 dogs referred to the Clinic of Parasitic Diseases from the Faculty of Veterinary Medicine of Iasi with similar symptoms to canine babesiosis. After recording data regarding age, breed and gender of all dogs and the clinical examination of the patients, two peripheral blood samples were collected from each patient for the following investigations: Diff-quick stained blood smears and blood tests (hematological, biochemical, serologic). After analysis of blood smear, all the dogs (42/42) were positive for *Babesia* spp. and the most common clinical signs identified were: fever – 37/42 (88,1%), pale mucous membrane – 31/42 (73,8%) and hemoglobinuria – 31/42 (73,8%). The results of hematologic tests revealed thrombocythopenia – 40/42 and moderate to severe anemia. Further serological tests detected *Babesia gibsoni* antibodies in 2/42 blood samples. Depending on the results of the blood tests, the therapeutic dose of imizole was administered in a single dose or divided into two doses, administered within a maximum of 12 hours. In conclusion, the present study emphasizes the importance of paraclinical investigations in order to identify possible co-infections and adjust treatment in infected dogs.

Keywords: canine babesiosis;diagnosis; treatment;

INTRODUCTION

Canine vector-borne diseases include several types of pathogens (bacteria, parasites, viruses) that cause a variety of health problems for dogs (Irwin P.J., 2014). In Romania, one of the most important vector-borne diseases is represented by babesiosis. Canine babesiosis is caused by a protozoan from the genus Babesia, transmitted through many species of ticks (Grey J.S. et al., 2019). There are several, genetically different species of babesia: large where merozoites measure between 3-5µm (Babesia canis canis, Babesia canis vogeli, Babesia canis rossi) and small, where merozoites measure between 1,5-2,5 µm (Babesia conradae, Babesia gibsoni, Babesia microti-like) (Imre M. et al., 2013; Tudor P. et al., 2008). Dermacentor reticulatus and Ixodes ricinus are the species of ticks incriminated for the development of the disease in the Eastern part of Romania. The increase in spreading of arthropod vectors globally, as well as the association of canine vector-borne diseases can be explained by the change in climatic and ecological factors but also by the increase of the mobility of humans and animals, thus determining the global spread of canine vector-borne diseases (Andersson M., et al., 2017).

Vector diseases are considered real challenges for veterinarians, as clinical signs can sometimes be diffuse or overlapping with other vector-borne pathogens (Andersson M., et al., 2017; Grey J.S. et al., 2019). The diagnosis of babesiosis in dogs is based on corroborating the anamnesis, clinical signs (pale mucous membranes, hemoglobinuria, hyperthermia, icterus) and identification of intraerythrocytic piroplasmas in the cytological blood smears (Ionita M., et al., 2012).

MATERIALS AND METHODS

This study included 42 dogs suspected of babesiosis based on the anamnesis and clinical signs. After general clinical examination of the patients and transcription of medical history, two peripheral blood samples (EDTA and Clot activator) were collected from each patient for the following investigations: microscopic examination of the blood smear, hematological test, blood biochemical examination and two serological tests: SNAP 4DX Plus (IDEXX), respectively Babesia gibsoni antibody test (WellTest).

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Blood collection can be performed in two ways, in order to highlight hemoparasites: in vacutainer tubes, from the peripheral veins (brachycephalic, saphenous or jugular) or the capillary circulation (from the nose) (only for blood smears).
The smears are stained immediately after drying and fixation, to reduce the risk of erythrocyte modification. We used Diferential Quick stain kit (Modified Giemsa) according to the manufacturer's instructions for identification of babesia merozoites.

After confirmation of the diagnosis, the two blood samples collected were analyzed to determine the degree and the type of anemia, as well as to check the renal and hepatic function, frequently affected in canine babesiosis. These indicators are very important for adjusting the optimal therapeutic protocol in order to recover patients. The serum has also been used to check for possible co-infections, often found in canine tick-borne diseases. Therefore, we chose to use the SNAP 4DX Plus (IDEXX) test, for identification of 4 possible pathogens: Ehrlichia canis, Anaplasma platys/Anaplasma phagocythophillum, Borrelia burgdorferi and Dirofilaria immitis while for the detection Babesia gibsoni, we used Babesia gibsoni antibody test (WellTest). Both rapid tests were used according to the manufacturers' instructions.

RESULTS AND DISCUSSION

The results of the study showed that the most affected dogs were common or mixed breed - 40.48% (17/42), followed by the Pekingese - 11.9% (5/42), and with 7,14% (3/42): Husky, German Shepherd and Caucasian Shepherd.

Dogs can become infected with *Babesia spp.* regardless of age. It can be seen in Table 1 that the differences between the number of cases in different age categories are small.

Regarding the sex of dogs, the number of infestations in males was higher (66.66%) compared to females (33.34%).

According to the data obtained from the anamnesis reported in Table 2, we found that 76.19% (32/42) of the dogs participating in the study were not externally dewormed according to the recommendations of veterinarians. The lack of awareness of the owners by postponing external deworming, leads to an increase in the cases of vector-borne diseases, which can be fatal in some situations.

Among the patients included in this study, 88.1% of the dogs were brought in consultation for apathy, 83.3% for lack of appetite and 73.8% for the presence of dark urine.

Clinical examination is very important for an accurate diagnosis. The clinical signs in canine babesiosis are not specific, but the data from the anamnesis corroborated with the clinical examination and the cytological examination of the blood, are sufficient for diagnosis.

In babesiosis, the main clinical signs are: lethargy, depression, fever, pale mucous membranes, jaundice, hemoglobinuria, splenomegaly, hepatomegaly. The most common clinical signs found in the 42 dogs studied were: fever - 37/42 (88.1%), pale mucous membranes - 31/42 (73.8%) and hemoglobinuria - 31/42 (73, 8%), followed by dehydration and jaundice (Figure 1). Morphological examination of the blood smears is the fastest method of diagnosis. It is also often used due to its low cost.

To confirm parasitic infestation with small species of *Babesia spp. (Babesia gibsoni, Babesia microti)* the definite diagnosis is given only by using molecular biology techniques. For all blood samples collected, between 3-5 smears were performed, and at least 50 microscopic fields were examined x100 objective. All blood samples were positive for *Babesia canis* (Figure 2).

Due to the pathogenesis, the most important change in the blood count is found in the red blood cells, as canine babesiosis often develops with moderate to severe anemia. Thus, red blood cells, hemoglobin and hematocrit will have low values and following the hemorrhagic diathesis, a decrease in platelets can also be observed. These aspects can also be noticed in the case of the 42 dogs studied, presented in Table 3, where 30/42 of the patients had low red blood cell counts, and 34/42 had hematocrit below normal limits. In patients with hematological values within normal limits, the degree of parasitaemia was low and the clinical signs were unstable. Thrombocytopenia was found in 40/42 canine patients.

Analyzing the 42 biochemical blood results, we found increased values of renal parameters (BUN - 24/42), liver enzymes (GGT - 25/42), alkaline phosphatase (29/42) and total bilirubin (22/42). Also, Table 3 shows normal values of creatinine (CRE - 32/42) or alanine aminotransferase (ALT - 25/42), which led to a faster recovery of patients after imizole administration.

The 42 serum samples were analyzed using the SNAP 4DX (IDEXX) and *Babesia gibsoni* Atc rapid tests (WellTest) to identify possible co-infections with other pathogens transmitted through ticks.

Table 1 - Prevalence of positive cases of canine babesiosis in relation to breed, age and sex

	Number of positive dogs/total dogs	%
Breed		
Mixed/common breed	17/42	40,48%
Husky	3/42	7,14%
Pekingese	5/42	11,9%
Labrador	1/42	2,38%
Shar-pei	1/42	2,38%
English Setter	1/42	2,38%
Caucasian Shepherd	3/42	7,14%
Samoyed	2/42	4,76%
Bichon	2/42	4,76%
Akita Inu	2/42	4,76%
Yorkshire terrier	1/42	2,38%
German Shepherd	3/42	7,14%
Bernese Shepherd	1/42	2,38%
Age		
0-1 year	7/42	16,7%
1-5 years	12/42	28,6%
5-7 years	11/42	26,2%
7-15 years	12/42	28,6%
Sex		
Females	14/42	33,34%
Males	28/42	66,66%

% - percentage

Table 2 - The informations obtained from the owners (the anamnesis)

	Number of positive dogs/total dogs	%
Without external deworming	32/42	76,19%
Apathy	37/42	88,1%
Inappetence	35/42	83,3%
Dark urine	31/42	73,8%
Ticks found on the body / observed by the owner and removed	28/42	66,7%
Vomiting	18/42	19,05%
Locomotor problems	5/42	11,9%
No medical history	10/42	23,81%

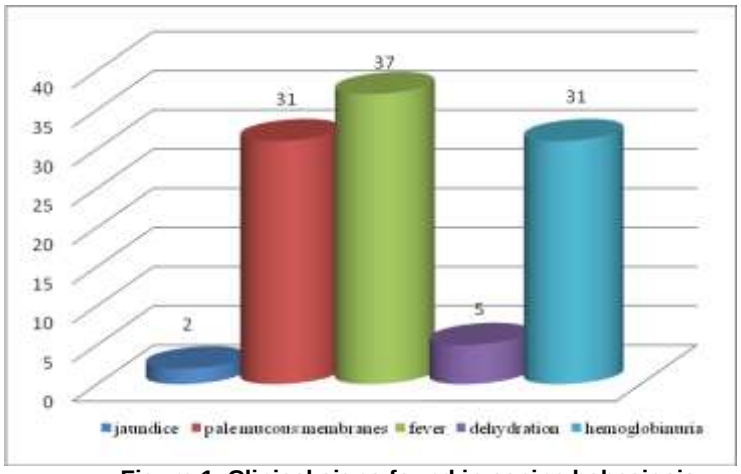

Figure 1 Clinical signs found in canine babesiosis

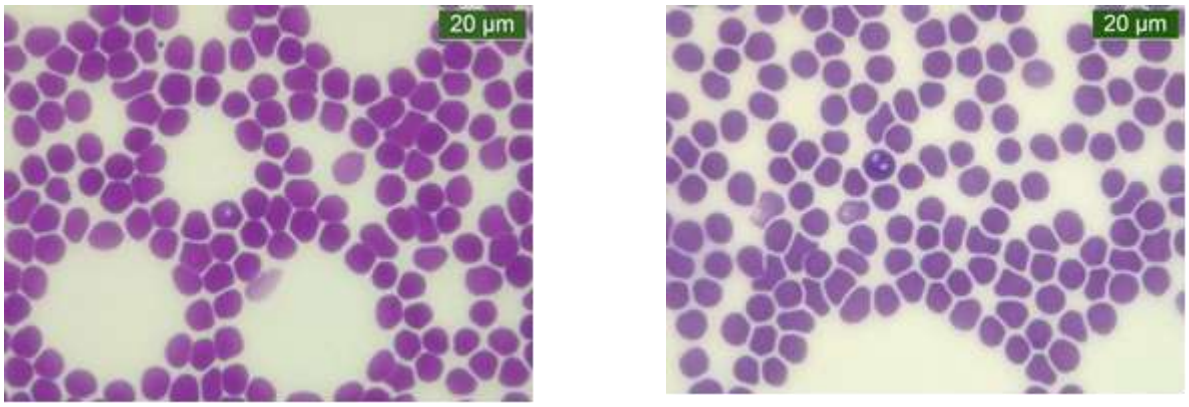

Figure 2 Babesia canis – blood smear, x100

Table 3 - The results of blood tests

Parameter	Low values	Normal values	High values	Total number of analyzes
		Hematological examination		
WBC	22	16	4	42
LYM	19	22	1	42
MON	11	31	0	42
NEU	12	27	3	42
EOS	0	32	10	42
BAS	0	42	0	42
RBC	30	11	1	42
HGB	25	15	2	42
HCT	34	8	0	42
MCV	7	35	0	42
MCH	2	28	12	42
PLT	40	2	0	42
		Biochemical examination		
ALP	0	13	29	42
ALT	0	25	17	42
TBIL	0	20	22	42
BUN	2	16	24	42
CRE	0	32	10	42
TP	17	25	0	42
GLOB	20	21	1	42
GLU	0	19	23	42
GGT	5	12	25	42

Corroborating the results of serological tests, we found the presence of *Babesia gibsoni* antibodies in 2/42 blood samples (Figure 3) and 0/42 positive samples for infections with *Ehrlichia* spp., *Anaplasma* spp. or *Borrelia* spp.

Figure 3 *Babesia gibsoni* Atc. test - positive

Therapeutic protocol

All patients diagnosed with canine babesiosis entered the following protocol, after receiving the written consent of the owners:

- Rehydration with NaCl, depending on the degree of dehydration;
- Administration of atropine sulfate (Atropine sulfuric 1%), in a dose of 0.1-0.2 ml and waiting 10 minutes or hydrocortisone hemisuccinate;
- Administration of Imidocarb dipropionate (Imizol) at a dose of 6.6 mg / kg (Table 4);
- 30 minute monitoring in case of side effects;
- Nutritional supplements, vitamins.

Table 4 - Therapeutic protocol in canine babesiosis

Activ substance	Dose (mg/kg)	Route of adm.	Interval (hours)	Duration of treatment (days)	Pathogen	
					B. canis	*B. gibsoni*
Imidocarb dipropionate	5-6,6 7,5	IM/SC IM/SC	1 adm. 1 adm.	Repeat in 14 Not necessary	+++	+
Diminazene aceturate	3,5-5	IM	1 adm.	Not necessary	+++	++
Azithromycin and Atovaquone	10 13,3	PO PO	24 8	10 10	+++	+++
Clindamycin and Doxycycline and Metronidazole	25 5 15	PO PO PO	12 12 12	90 90 90	+	+
Phenamidine isethionate (Lomadin, Fenamidin)	15-20	SC	24	2	+++	++

References: *Sykes, J. E., 2013*; *Greene, C. E., 2012*
Adm. - Administration

Some dogs may develop a number of side effects, such as parasympathetic symptoms after administration of imidocarb dipropionate (Imizol): sialoree, vomiting, general weakness. These symptoms can be prevented by administering a dose of atropine sulfate.

Depending on the results of blood biochemical tests, the therapeutic dose of imizole was administered in a single dose or in two divided doses, administered within a maximum of 12 hours.

The fractional therapeutic dose was administered only in the following cases: geriatric patient, liver or kidney failure, gestation or patients with chronic pathologies.

Improving of the general condition of positive patients with babesiosis usually occurs in 24-72 hours, but some dogs can heal in 4-7 days. Continuous monitoring of hematological parameters is performed until they return to normal limits.

In dogs infested with Babesia gibsoni, the PCR test should be performed 60-90 days after treatment with atovaquone and azithromycin (Irwin P.J., 2014).

CONCLUSION

The study was performed on a group of 42 canine patients suspected of babesiosis, which were presented to the Medical Clinics and Parasitic Diseases Clinics within the Faculty of Veterinary Medicine Iasi. The most affected dogs were those of common or mixed breed - 40.48%, followed by the Pekingese - 11.9%, regardless of age. The most common clinical signs were: fever - 37/42 (88.1%), pale mucous membranes - 31/42 (73.8%) and hemoglobinuria - 31/42 (73.8%).

We observed changes in hematological tests - moderate to severe anemia (decreased red blood cells, hematocrit and hemoglobin) and thrombocytopenia - 40/42 canine patients; changes in renal and hepatic parameters, resulted from the biochemical blood examination. We detected Babesia gibsoni antibodies in 2/42 blood samples positive at canine babesiosis, which showed a moderate form of infestation with Babesia canis and responded to treatment with Imidocarb dipropionate.

Depending on the results of biochemical blood tests, the therapeutic dose of imizole was administered in a single dose or in two divided doses, administered within a maximum of 12 hours.

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