Studies regarding the clinical management optimization in babesiosis in dogs

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Abstract

The diagnosis of canine babesiosis is based, most of the times, on the patients' clinical signs, correlated with the ticks' parasitism, the emergence season, but the most important clue is performing the laboratory exams.The manifestations of the disease had, in most of the studied individuals, as a characteristic, the well-known clinical tetrad: fever, icterus, anemia and hemoglobinuria, mentioning that for the diagnosis confirmation it was necessary to perform the blood smear microscopic examination and to detect the intracellular parasites or the damaged erythrocytes.In the group of dogs with babesiosis, the haematological examination revealed a distinctly significant (p<0.01) decrease of the mean number of erythrocytes, indicating a severe haemolytic anemia caused by their massive destruction due to the parasites' mechanical and toxic actions, that along with the destruction of the immature erythrocytes (reticulocytes) - an indicator of regenerative erythropoiesis, ultimately causes tissue hypoxia. There is also found a statistically significant decrease (p<0.05) of the mean values of the haemoglobin and haematocrit. In dogs with babesiosis was noticed a statistically significant increase (p<0.05) of the urea's mean value and an increase (but not significant - p>0.05) of the creatinine's mean value, compared with those of the control group. Thus, a major role in the pathogenesis of the renal function impairment in dogs with babesiosis can be attributed to the septic-toxic state induced by the intraerythrocytic parasites. The mean value of the bilirubin in the group of dogs with babesiosis was distinctly significant higher (p<0.01) compared to the control group, a sign of massive erythrocytes' degradation, which stands at the basis of the haemolytic anemia diagnosis and thus the liver function decrease, translated by the significant increase (p<0.05) of the hepatocellular injury specific enzymes activity (ALT). Another biochemical change was hypoglycaemia. In this context, the association between severe anemia and hypoglycaemia may be explained by the effect of hypoxia on the glycolysis and by the fact that the severity of the anemia is closely related to the disease severity and the inflammatory response intensity, which may lead to an intensification of metabolic processes and, consequently, to an excessive glucose consumption.

Key words: babesiosis, dogs, haematological and biochemical exams

Introduction

Due to the two factors involved, the host organism (of different breeds and ages, at which different collateral conditions may or may not be present) and the parasite (of different species, three identified in Romania), babesiosis develops and expresses a highly complex and intricate clinical-pathogenically and paraclinical ensemble (*10*).

The purpose of this paper is to contribute to the identification and integrated assessment of the epidemiological framework and coordinates of babesiosis on an delimited area in Ilfov county and to evaluate and subsequently appreciate in an integrated manner the prognostic values of the variations of some haematological and biochemical parameters.

As mentioned in the speciality literature, the diagnosis of canine babesiosis is most often based on the clinical signs of the patients, correlated with tick parasitism, season of onset (*8,9,11*), but the most important clue is represented by the performing of the laboratory exams (*6,7*).

The haematological abnormalities observed during the acute form of the disease were also mentioned by other authors (*14*), who point out that the major mechanism of producing serious

imbalances in the body is ischemia, because a very large number of erythrocytes that are parasitized are retained and destroyed in the spleen.

In this context, the body cannot produce new erythrocytes at the rate at which they are destroyed and, thus, increases the number of reticulocytes - an indicator of regenerative erythropoiesis (*3,12,13*), which leads to irreversible changes at the liver, splenic or renal level (even with lungs or cardiac complications).

Regarding the symptoms of babesiosis in the acute form, they were generally uniform and translated, in most cases, by apathy, fatigue, anorexia, loss of appetite, febrile syndrome characterized by sudden hyperthermia (39.8-41ºC), anemia, jaundice, hemoglobinuria, respiratory disorders (*2,5*).

It is worth mentioning the fact that, in most cases, the manifestations of the disease had, as a characteristic, the well-known clinical tetrad, represented by fever, icterus, anemia, hemoglobinuria, also mentioning the fact that in order to confirm the diagnosis, the microscopic examination of a blood smear of peripheral blood and highlighting intracellular parasites or the damaged erythrocytes were necessary (*1*).

Materials and methods

The present study was performed on a number of 67 dogs belonging to several breeds, of different ages and genders, presented and consulted at the Konivet Medical Veterinary Clinic, Domneşti commune, Ilfov county, but also at the clinic within the Faculty of Veterinary Medicine Bucharest, from 2016 until 2018.

It is worth mentioning the fact that, due to the quite large variations of the haematological and biochemical parameters that exist between different breeds of dogs, for the accuracy of the results and for a more accurate statistical interpretation, the patients in the control group were selected in such a manner that, they would largely belong to the same breeds as the patients with babesiosis.

The dogs in the group suspected of babesiosis, underwent rigorous physical and clinical examination. During the clinical examination, in the majority of the infested dogs (and included in the studied group), the presence of ticks could be observed on the skin.

Since the identification of endoglobular parasites in the blood smear represents the decisive element of the etiological diagnosis, the infested dogs were included in the studied group after at least two smears of peripheral blood (*May-Grumwald* coloured) were performed in order to detect the *Babesia spp.* parasites from the red blood cells.

In order to confirm the diagnosis and establish an individualized therapeutic protocol in patients with suspicion of babesiosis, it was preceded to harvesting biological samples (whole blood on EDTA) for haematological examination (to determine the degree of anemia) and biochemical exams (to determine the degree of impairment of the hepatic and renal functions).

Anamnestic data and disease history are an important component in the preparation and implementation of the protocol and the clinical examination chart, which is why the elements that are revealed from the (detailed) anamnesis may highlight various pre-existing or competitive conditions, as well as the clinical signs, but also the reason for presentation at the consult and medical evaluation.

The haemogram, which is a basic screening test, associated with examination of the blood smear, provide valuable information about the anemia degree and more. For the complete blood count, blood was harvested (by venous puncture) in special tubes with anticoagulant.

The automatic analyser used to perform the haematological determinations was Melet Schloesing MS 45, and for the determination of the biochemical parameters it was used the

Spotchem EZ 4430 ARKRAY analyser, a dry biochemistry analyser that uses as a principle for the parameters determination the optical measurement of the reflection intensity (*4*).

Results and discussion

Dogs infested with *Babesia spp.* present a clinical picture with various manifestations of the disease, the incubation period being comprised between 7 and 14 days, but depending on the pathological condition of the patient, the clinical signs of the disease can appear even from the 5th day of infestation.

The manifestations of the disease had in most of the studied individuals, as a characteristic, the well-known clinical tetrad, represented by fever, jaundice, anemia, hemoglobinuria, mentioning that for the confirmation of the diagnosis it was necessary the microscopic examination of a peripheral blood smear and to highlight the intracellular parasites in order to confirm the diagnosis. The clinical tetrad (fever, jaundice, anemia, hemoglobinuria), specific to canine babesiosis, is well represented also in the studied group. Thus, 84.2% (n=16) of dogs with babesiosis had a rectal temperature above $39,2^0$C (Chart 1).

Rectal temperature (degrees Celsius) in patients with babesiosis

15,7

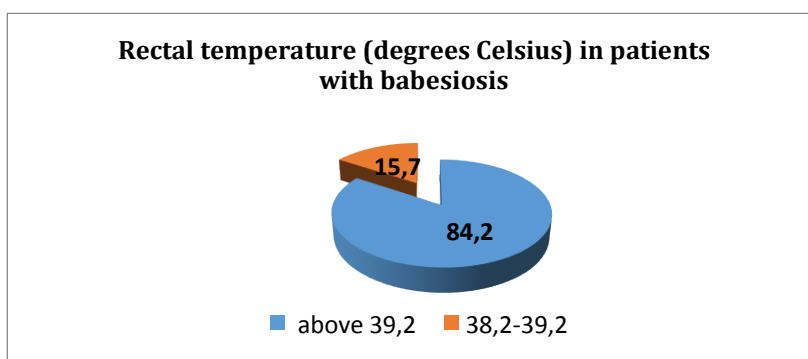84,2

■ above 39,2 ■ 38,2-39,2

Chart 1. Representation of the percentage of dogs
with babesiosis that presented fever

Also, it can be mentioned the fact that 47.3% of the dogs from the group with babesiosis presented at clinical examination jaundice at the level of the mucous membranes and/or the abdominal region skin, and more than half of them (57.8%) also presented hemoglobinuria (Chart 2). Noteworthy is the fact that a very large percentage of the dogs with babesiosis, of the studied group, (89.4%) presented paleness of the mucous membranes.
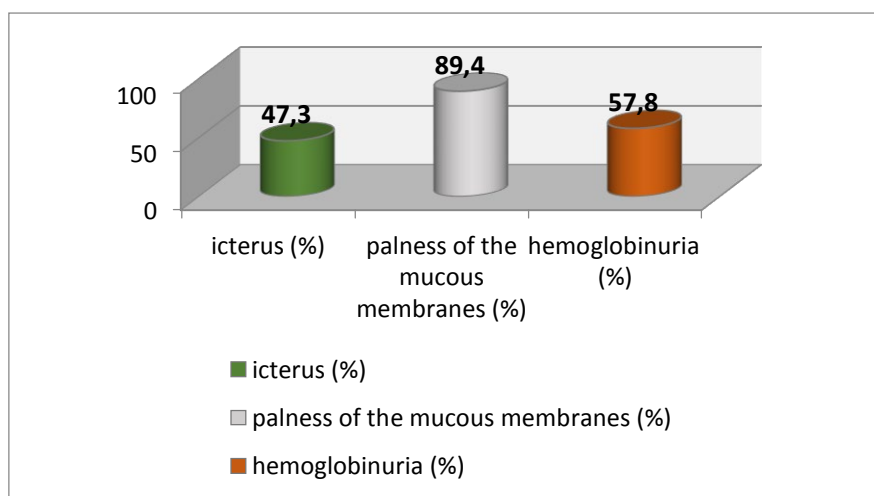
Chart 2. Representation of the percentage of dogs with babesiosis which
presented different clinical signs

Following the comparative analysis of the haematological and biochemical parameters in dogs from the babesiosis group and of those from the control group, significant differences were observed, as other authors have also pointed out (*5*).

In this context (Table 1), in dogs from the group with babesiosis, the haematological examination revealed a distinctly significant decrease (*p<0.01*) of the average number of erythrocytes (4.4 ± 0.8 mil/mm^3), compared to the control group (6.45 ± 0.9 mil/mm^3), which denotes a severe haemolytic anemia caused by the massive destruction of the red blood cells following the mechanical and toxic action of the parasites, which ultimately causes tissue hypoxia due to the massive destruction of the red blood cells, but also of the immature erythrocytes (reticulocytes) - an indicator of regenerative erythropoiesis, which get into circulation under the action of erythropoietin (*14*).

In dogs from the babesiosis group (Table 1), was observed a statistically significant decrease (*p<0.05*) of the mean values of haemoglobin (9.1 ± 0.3 g/dl) and haematocrit ($30.4 \pm 6.4\%$), compared with the average values of these parameters in the control group (13.8 ± 1.0 g/dl, respectively, $39.7 \pm 2.4\%$).

Table 1. The mean values of the haematological and biochemical parameters in patients with babesiosis

No.	PARAMETER	The mean values in the control group *(n=13)*	The mean values of the patients with babesiosis *(n=19)*	The limit values recorded in the patients with babesiosis *(n=19)*
1.	**Erythrocytes** *(mil/ mm³)*	6.45 ± 0.9	4.4 ± 0.8***	3.8-5.0
2.	**Haemoglobin** *(g/dl)*	13.8 ± 1.0	9.1 ± 0.3**	8.5-9.7
3.	**Haematocrit** *(%)*	39.7 ± 2.4	30.4 ± 6.4**	26-34
4.	**Total leukocytes** *(mii/mm³)*	9.2 ± 0.8	8.6 ± 0.4*	8.0-9.2

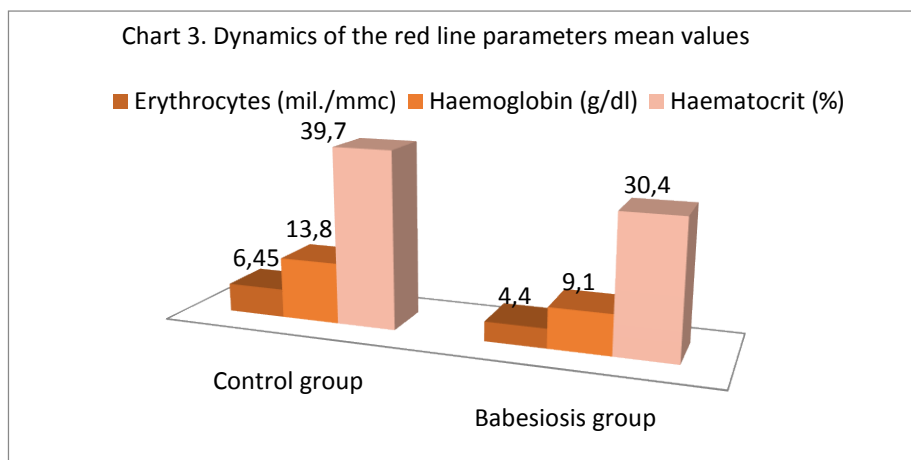|					
5.		**Neutrophils** *(%)*	58 ± 7	41 ± 2**	37-45
6.	**Leukocytes formula**	**Lymphocytes** *(%)*	30 ± 8	43 ± 2**	39-47
7.		**Monocytes** *(%)*	7 ± 4	10 ± 3*	7-13
8.	**Bilirubin** *(mg/dl)*		4.04 ± 0.2	3.94 ± 0.2***	0.8-11.2
9.	**Total proteins** *(g/dl)*		6.7 ± 0.21	5.4 ± 0.24*	4.9-5.9
10.	**Albumins** *(g/dl)*		3.1 ± 0.05	2.3 ± 0.08**	2.0-2.6
11.	**Globulins** *(g/dl)*		3.6 ± 0.2	3.1± 0.4*	2.7-3.4
12.	**A/G ratio**		0.86 ± 0.1	0.73 ± 0.10*	0.61-0.86
13.	**Glucose** *(mg/dl)*		119.6 ± 6.2	87.4 ± 5.0**	52.0-101.4
14.	**ALT** *(U/l)*		17.4 ± 1.5	51.0 ± 8.0**	37-65
15.	**Urea** *(mg/dl)*		15.6 ± 0.9	24.8 ± 2.2**	19-31
16.	**Creatinine** *(mg/dl)*		0.9 ± 0.06	1.4 ± 0.1*	0.8-2.0

* p>0,05 – no significant differences
** p<0,05 – significant differences
*** p<0,01 – distinctly significant differences

Worth mentioning is the fact that the clinical manifestations described in the group of dogs with babesiosis, together with the significant decreases in the parameters of the red line (Chart 3) - are characteristic of a severe haemolytic anemia, with the classic symptoms of intravascular erythrolysis being met: jaundice, paleness of the mucous membranes and fever.

The changes with statistical significance of the red line indicate the fact that the major mechanism of producing some serious imbalances in the body is ischemia, because a very large number of erythrocytes that are parasitized are retained and destroyed in the spleen.

Chart 3. Dynamics of the red line parameters mean values

■ Erythrocytes (mil./mmc) ■ Haemoglobin (g/dl) ■ Haematocrit (%)

39,7

13,8

6,45

30,4

4,4   9,1

Control group

Babesiosis group

In the studied group of dogs, there is found also a statistically significant increase (*p<0.05*) of the mean value of urea (24.8 ± 2.2 mg/dl), but also an increase (not significant - *p>0.05*) of the mean creatinine value (1.4 ± 0.1 mg/dl), compared with the mean values of the control group (15.6 ± 0.9 mg/dl, respectively 0.9 ± 0,06 mg/dl), this fact being the consequence of the septic-toxic state

induced by the parasites, in addition to the massive purge of haemoglobin and bilirubin, with direct toxic effect on the nephrons (Chart 4 and Table 1).
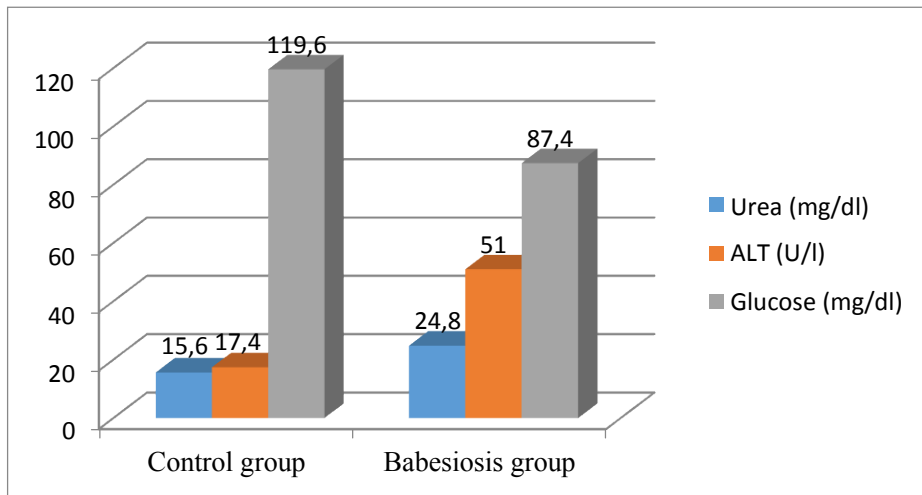

Chart 4. The mean values of urea, ALT and blood glucose in dogs from the control and babesiosis groups

Also, we notice the appearance of some statistically significant changes also in the case of the enzymes with specificity for hepatocellular lesions (Chart 4), with an increase ($p<0.05$) of the ALT activity in dogs with babesiosis (51.0 ± 8.0 U/I) compared to the mean value of the control group (17.4 ± 1.5 U/I).

Following the dosing of bilirubinaemia, it was found that the mean value of this parameter is distinctly significant higher ($p<0.01$) in the group of dogs with babesiosis (3.94 ± 0.2 mg/dl), compared to the mean value of the control group (0.3 ± 0.2 mg/dl), being a sign of the massive degradation of the erythrocytes that stands at the basis of the diagnosis of haemolytic anemia, having as a consequence the decrease of the hepatic function. Hypoglycaemia (87.4 ± 5.0 mg/dl) detected in patients with babesiosis can be attributed to and correlated with the effect that the hypoxia had on the glycolysis (Table 1).

Conclusions

1. In the studied group of dogs with babesiosis, the decreases (with statistical significance) of the red line parameters, corroborated with the clinical manifestations that constitute the specific tetrad of this hemosporidiosis - are characteristic of a severe haemolytic anemia, caused by the massive destruction of the erythrocytes consecutive the specific action (mechanical and toxic) of the parasites, and bring together the symptomatic assembly of intravascular erythrolysis: anemia, icterus, hemoglobinuria and fever.

2. The analysis of the complete blood count in dogs from the group with babesiosis, highlighted a distinctly significant decrease ($p<0.01$) of the average number of erythrocytes and also a statistically significant decrease ($p<0.05$) of the mean values of haemoglobin and haematocrit compared with the control group.

3. The clinical manifestations specific to the babesiosis had a general character, observing the fact that 47.3% of the patients had jaundice of the mucous membranes and / or of the hairless and depigmented skin, 57.8% had hemoglobinuria, 84.2% showed a hyperpyrexia background -

body temperature over 39.2^0C, and at a high percentage (89.4%) there was appreciated an obvious paleness of the apparent mucous membranes (correlated with the intensity of the haemolysis).

4. The results of the biochemical examination in the patients with babesiosis revealed the presence and intensity that can be correlated with the presence and the degree of toxic-septic states (induced by intraerythrocytic parasites), in the pathogenesis of the renal and / or hepatic function.

5. The biochemical parameters which attest the degree of functioning and the renal morphofunctional integrity undergo significant changes, evidenced by the statistically significant increase ($p<0.05$) of the mean value of the urea (24.8 ± 2.2 mg/dl), in conjunction with an increase ($p>0.05$) of the creatinine mean value (1.4 ± 0.1 mg/dl).

6. The distinctly significant increases ($p<0.01$) of the average values of bilirubinaemia (3.94 ± 0.2 mg/dl) in the individuals with babesiosis, compared to those registered in the control group, certify the dominant pathogenetic degradation of the red blood cells, constituting a preliminary and early diagnosis criteria of haemolytic anemia (including in the subclinical forms), with aggressive repercussions that have the effect of altering the liver function and a direct toxic effect on the nephrons, as a result of the massive clearance of haemoglobin and bilirubin at this level.

7. The identification of hypoglycaemia (87.4 ± 5.0 mg/dl) in patients with babesiosis can be attributed and correlated with the effect of the hypoxia on the glycolysis, a biochemical modification that can be associated with the degree and intensity of the anemia, jaundice and vascular collapse, in correlation with the rest of the lesions identified and associated with the inflammatory response, as well as the modifications/ alterations of the carbohydrate metabolism.

8. The blood cytomorphological examination (blood smear) in patients with babesiosis revealed a variable number of intraerythrocytic *Babesia spp.* parasites, (depending on the degree of impairment and the moment when the patient was brought at the veterinarian), constituting the element that allows the obtaining of a diagnosis of certainty (etiological) and identification, assessment and quantification of the morphological changes of the red blood cells (mainly anisocytosis and poikilocytosis), which undoubtedly attests the intense demand of the haematogenous marrow (in the process of erythropoiesis), indicating and attesting the regenerative character of the anemia.

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