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Original article

Cardiac disorders in dogs infected with *Babesia canis*

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Abstract

The aim of this study was to assess cardiac disorders in dogs infected with *B. canis*. The study included 50 dogs with babesiosis and 20 healthy control animals. All the animals had haematological tests, ECG, echocardiography and serum troponin I and CK-MB levels checked. The haematology in the group of dogs with babesiosis confirmed thrombocytopaenia in 100% of dogs, decreased haematocrit in 52% and anaemia in 46%. The most common abnormalities in ECG and echocardiography in dogs infected with protozoa included: change in appearance and/or amplitude of the T-wave (34%), increased fractional shortening (24%), an increased sinus rhythm (14%) and heart axis deviation (10%). In 19 of the 50 dogs with babesiosis, the level of serum troponin I was elevated. In 2 dogs that died from babesiosis, the troponin level I was very high. The ECG confirmed sinus tachycardia and interpolated ventricular beat in these animals. In all dogs with babesiosis that were used in the study, the serum CK-MB was high or very high and was within limits of 23.17 U/L – 369.62 U/L. The highest kinase concentration (367.33 U/L and 369.62 U/L) was observed in dogs that died due to the disease. The presented results prove that cardiac changes are common in canine babesiosis, but that most changes are nonspecific and appear to have little clinical significance. Cardiovascular assessment should be based on the assessment of the level of troponin I and CK-MB in the serum of sick animals. High concentrations of these factors might be indicators of poor prognosis.

Key words: *Babesia canis,* dogs, cardiac changes

Introduction

Canine babesiosis is a common and clinically significant tick-borne disease caused by haematozoan parasites of the genus *Babesia* (Mehlhorn et al. 1980, Heile et al. 2006, Adaszek et al. 2009). The classification of *Babesia* spp. places them in the order of Piroplasmida within the phylum Apicomplexa. Two morphologically distinct forms of the erythrocytic stage in the canine host were recognized in early studies that led to the naming of the larger form, measuring approximately 3-5 µm as *B. canis*, and the smaller (1-3 µm) as *B. gibsoni* (Adaszek et al. 2010). On the basis of cross-immunity, serological testing, vector

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specificity and molecular phylogeny, *B. canis* was reclassified into three sub-species (*B. canis*, *B. rossi* and *B. vogeli)*. All of them are now considered to be separate species (Zahler et al. 1998, Carret et al. 1999, Costa-Junior et al. 2009). Within the small piroplasms, three distinct species are currently recognized to cause disease in dogs: *B. gibsoni*, *B. conradae* and a *Babesia microti*-like piroplasm (*Theileria annae*) (Conrad et al. 1991, Camacho et al. 2001, Kjemtrup and Conrad 2006, Irvin 2009).

In our earlier works we have described the clinical course of babesiosis in dogs from areas of eastern Poland and disorders regarding selected haematological and biochemical parameters of the serum of dogs suffering from this disease (Adaszek et al. 2009). The clinical picture of dogs suffering from babesiosis due to infection with *B. canis* parasites is diverse, ranging from hyper-acute to acute and finally chronic types of disease (Schetters and Eling 1997, Adaszek et al. 2009).

The main cause of death in the course of babesiosis is anoxia and shock (Bourdoiseau 2006). According to our own observations, the deaths of the dogs in which the disease was diagnosed and treatment started are more frequently caused by complications associated with renal and hepatic insufficiency accompanied by elevated levels of serum urea and bilirubin (Adaszek et al. 2009).

Recently, more cardiovascular disorders in the course of babesiosis have been reported, which manifest in accelerated heart rhythm and dyspnoea. The aim of the study was to evaluate cardiac disorders in dogs infected with *B. canis* protozoa.

Materials and Methods

Animals used in the study

The study was carried out at the Clinic of Infectious Diseases, University of Life Sciences in Lublin between May and November 2015. It included 50 dogs infected with *B. canis* – confirmed by microscopic and molecular (PCR) tests – and 20 healthy dogs in a control group. The following criteria were applied for assigning the dogs to the groups: confirmed babesiosis and increased heart rate, number of breaths and signs of dyspnoea demonstrated in the clinical examination. The dogs in the control group were brought to the clinic for prophylactic vaccinations against infectious diseases. Animals of different breeds and sex were selected to the study, aged 2-8. Haematology tests, ECG, echocardiography was performed in these dogs as well as troponin I and CK-MB levels in serum of all animal were determined.

Haematological test

The haematological test was performed with Exigo VET analyser. The parameters assessed were as follows: erythrocytes count in the blood (n x 10^9), haematocrit (Ht) (%), haemoglobin (g/dL), total leukocytes count (n x 10^6) and thrombocytes count (n x 10^6).

Electrocardiography and echocardiography

The electrocardiography was performed with ASPEL AsCard Mr. Red 001v device in a standing position. The assessed parameters were: heart rate, axis deviation, and duration/amplitude of the individual constituents of the electrocardiogram (waves, segments and intervals). Electrocardiograms were recorded at a tape speed of 50 mm/s and an amplitude of 1 mV/cm.

The echocardiography was performed with ALOKA Prosound SSD-4000 (with probe Aloka Phased Array 2.1-3.8 MHz UST-5299) device on a subject in a standing position. The heart structure and function were assessed. Various imaging procedures were used, including two-dimensional imaging (2D), M-mode procedure and Doppler technique. The two-dimensional imaging covered long and short axis views from the right parasternal acoustic window and the left parasternal acoustic window. These views allowed for a quantity evaluation of the heart cavities and the heart's systolic function, and the measurement of the left atrium to left aorta proportion (LA/Ao). The M-mode echocardiography assessed the size of the left ventricle lumen, the thickness of the interventricular septum and a free wall of the left ventricle in the end-diastolic and end-systolic phases. The Doppler echocardiography evaluated laminar and possible turbulent blood flow through the aorta valves, the pulmonary valve and the bicuspid and tricuspid valves, and a quantitative evaluation of the speed and volume of the blood flow was also performed. This test was carried out before instituting intravenous fluid therapy.

Troponin I (cTnI) test

The level of troponin I in the plasma of the sick dogs was measured using the immunofluorescent method in a commercial laboratory. Directly after the blood was centrifuged, the serum for testing was frozen at -80°C and was transported to the laboratory.

Isoform of creatine kinase (CK-MB) tests

The level of creatine kinase isoform in serum of the blood was measured spectrophotometrically in the Mindray BS-130 analyser using the ACCENT-200 CK-MB (Cormay Poland) reagent.

Statistical analysis

To compare and to show correlations between the levels of troponin I, CK-MB and changes in the ECG the following statistical methods were used:

– Calculation of descriptive statistics such as the mean, deviation, standard deviation (SD), median (Me) and percentages.

– Use of tests: -χ2 test, the Shapiro-Wilk test, the Mann-Whitney test, the Kruskal-Wallis test.

The test function in Mann-Whitney test was marked as "Z" or "U", depending on the size of the group when the two groups of animals were compared, and the Kruskal-Wallis test was used to compare more groups. The test function in this test was marked as "H".

For easier statistical analysis of the results based on ECG changes, the dogs were divided into 3 groups: 0 – dogs with normal ECG record; 1 – dogs with slight changes in ECG (e.g. increased rhythm, elevated T wave); and 2 – dogs with significant changes in ECG (e.g. nodal tachycardia, additional ventricular beats, bradyarrhythmia).

The Mann-Whitney rank test was used to demonstrate the differences between the haematology test results and changes in the ECG and echocardiography test results. Changes were considered statistically significant at $p<0.05$. Correlations were calculated using the Spearman method. The correlation coefficient r was calculated for each pair of compared parameters. The Statistica 10.0 PL software was used for the calculations.

Results

Results of haematology tests

In the group of dogs with babesiosis, the haematological examination revealed a fall of haematocrit below 37% in 26 dogs, a fall of erythrocytes below 5.5×10^{12} (bottom limit of the norm) in 23 dogs, leucopoenia (WBC $< 6 \times 10^9$) in 28 dogs and leucocytosis (WBC $> 10 \times 10^9$) in 5 dogs (Table 1). In the control group, the haematology test results did not deviate from the physiological norms (Table 2).

Electrocardiography results

The ECG revealed the following disorders in dogs with babesiosis, confirmed by haematological and molecular tests: increased sinus rhythm in 7 animals, heart-axis deviation in 5 dogs, change in appearance and/or amplitude of T wave in 17 dogs, widened QRS complex in 3 dogs, atrioventricular block I (AV block I°) in 2 dogs, ventricular tachycardia in one dog and additional ventricular beats in 3 dogs (2 of these dogs had premature ventricular complex (VPs), and 1 had an interpolated beat). The only disorder confirmed by ECG in the control group was an increased sinus rhythm in 7 of the subjects (Tables 1 and 2).

Echocardiography results

The echocardiography confirmed an increased fractional shortening (FS%) in 12 dogs and a slight increase in heart blood flow in 1 dog (1.76 m/s) No changes in other sick dogs were observed echocardiographically. Similarly, no abnormalities in this organ were detected by echocardiogram in the control group (Tables 1 and 2).

Troponin I (cTnI) test results

In 28 out of the 50 dogs with babesiosis, the serum troponin I level was within the physiological range. The concentration of this enzyme in the serum of three dogs reached the upper limit of the range, i.e. 0.2 ng/dL, and in 19 dogs the serum troponin level was elevated, i.e. > 0.2 ng/dL. In 2 dogs that died of babesiosis, the level of troponin I was very high, reaching 14 and 26.7 ng/dL respectively. The ECG in these dogs confirmed sinus tachycardia, interpolated ventricular beat and ventricular tachycardia.

Creatine kinase (CK-MB) tests

In all animals with babesiosis, the level of CK-MB in serum was high or very high, ranging from 23.17 U/L to 369.62 U/L. The highest kinase concentration was reported in dogs that died due to the disease (367.33 U/L and 369.62 U/L).

Results of statistical analysis

The statistical analysis showed a significant correlations between the changes in ECG in dogs with babesiosis and the activity of CK-MB in their serum

Table 1. Results of hematology, electrocardiography and echocardiography in dogs with babesiosis.

No of the dog	Results of haematological examination					Results of electrocardiography								Results of echocardiography				
	RBC x 10^{12}	Hb g/dl	Ht %	WBC 10^9/l	PLT 10^9/l	HR	P (ms)	P (mV)	PQ (ms)	QRS (ms)	R (mV)	T (mV)	QT (ms)	LA/ /Ao	FS (%)	RVOT (ms)	LVOT (ms)	LViDd (mm)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	6.11	15.1	39.7	8.4	29	110	40	+0.2	135	61	+0.7	-0.6	300	1.12	38	0.88	1.46	47.3
2	5.54	13.7	35.1	3.4	52	85	31	+0.25	85	55	+1.6	-0.5	170	1.38	33	0.72	1.28	46.1
3	6.14	15.2	41.2	9.4	25	170	36	+0.19	85	60	+0.7	+0.3	200	1.28	**44**	0.98	1.57	31.2
4	5.69	14.7	39.3	4.4	51	140	34	+0.11	90	65	+0.67	+0.2	185	1.19	31	0.68	1.19	33.4
5	7.12	17.7	49.6	3.8	43	135	32	+0.22	75	55	+1.2	-0.4	180	1.36	37	1.14	1.39	29.3
6	6.4	15.5	42.1	2	31	150	39	+0.2	105	58	+1.65	-0.2	220	1.21	**45**	0.83	1.33	31.8
7	5.78	14.5	37.9	5.7	41	145	37	+0.23	100	51	+0.6	-0.5	190	1.29	28	0.59	1.23	36.8
8	6.86	16.2	44.4	8.9	24	130	39	+0.19	115	60	+1.55	+0.35	210	1.05	30	0.81	1.42	51.9
9	4.37	11.1	28.7	5.3	34	120	32	+0.17	105	59	+1.45	+0.4	225	1.19	35	0.75	1.47	43.5
10	5.79	13.7	34.1	4.8	51	150	34	+0.26	95	53	+1.35	+0.35	215	1.38	**46**	1.12	1.51	34.8
11	5.36	13.1	33.2	4.2	31	140	37	+0.13	80	62	+1.5	+0.4	220	1.32	**42**	0.84	1.66	52.3
12	6.54	15.2	39.9	6.8	79	165	40	+0.35	85	55	+0.85	-0.5	200	1.08	32	0.79	1.18	61.3
13	5.88	13.7	38.6	2.3	38	150	38	+0.21	95	61	+1.7	-1.5	250	1.27	36	0.74	1.41	39.8
14	1.7	4.6	11.1	4.6	8	200	39	+0.15	81	57	+0.6	-0.4	220	1.41	**44**	0.66	1.19	38.5
15	0.73	4.1	6.2	20.7	16	175	42	+0.32	115	50	+1.3	+0.45	240	1.45	**43**	1.27	1.59	43.9
16	6.07	15.2	40.2	3.7	85	140	37	+0.29	100	85	+0.6	-0.4	205	1.13	38	0.85	1.31	35.8
17	5.08	12.6	32.4	7.7	200	130	40	+0.21	115	60	+0.75	-0.5	200	1.52	33	0.91	1.48	64.8
18	5.91	14.1	37.7	9.8	27	120	29	+0.28	100	55	+1.0	-0.4	210	1.11	26	0.75	1.09	55.6
19	3.78	8.9	24.8	6.8	19	150	33	+0.34	95	53	+0.5	+0.3	220	1.44	**44**	1.25	1.46	31.7
20	5.5	12.7	33.1	3.9	19	135	38	+0.18	85	62	+0.8	-0.1	210	1.28	28	0.88	1.36	49.3
21	4.5	10	25.9	9.7	116	145	40	+0.15	105	57	+1.5	+0.5	180	1.01	30	0.68	1.05	50.4
22	4.98	11.2	30.5	3.4	124	130	40	+0.36	90	61	+2.2	+0.9	215	1.29	40	1.01	1.32	38.7
23	3.89	9.9	23.5	27.2	40	165	36	+0.1	85	78	+0.55	+0.5	210	1.5	**50**	1.39	**1.76**	44.1
24	3.97	10.5	25.2	6.1	31	125	29	+0.36	90	59	+1.2	+/- 0.45	190	1.24	25	0.84	1.33	42.3
25	5.67	13.9	37.4	8.5	21	100	38	+0.21	95	62	+1.5	-0.6	220	1.39	31	0.98	1.52	55.4
26	6.32	15.1	40.3	6.7	39	175	40	+0.27	75	67	+1.1	-0.55	185	1.19	29	0.76	1.19	39.4
27	4.53	10.2	26.7	2.3	35	110	38	+0.33	85	61	+0.7	-0.25	210	1.43	28	0.62	0.99	49.7
28	4.75	11.7	29.7	5.6	28	125	36	+0.31	100	55	+1.1	+0.35	200	1.08	36	1.02	1.52	53.7
29	4.3	9.8	25.6	9.3	39	90	40	+0.22	100	58	+0.7	-0.2	195	1.18	31	0.85	1.29	51.2
30	6.95	16.2	43.6	5.6	30	145	30	+0.28	105	60	+1.1	+0.4	230	0.99	32	0.91	1.33	29.4
31	5.21	12.6	32.3	6.1	45	185	33	+0.27	85	51	+1.0	-0.5	205	1.38	**47**	1.28	1.66	49.4
32	6.45	14.9	38.1	5.3	73	145	31	+0.33	90	55	+0.5	-0.25	180	1.24	**42**	1.31	1.59	50.3
33	5.86	14.4	39.4	5.8	21	135	38	+0.22	90	53	+1.5	+0.6	175	1.13	29	0.84	1.31	35.7
34	3.65	5.4	15.4	32.6	17	220	41	+0.31	100	59	+1.1	-0.35	240	1.36	**45**	1.15	1.48	57.8
35	5.1	12.1	31.4	9.2	32	140	35	+0.19	105	61	+1.2	-0.4	200	1.32	36	0.98	1.25	49.8
36	6.5	14	38.1	5.6	36	135	37	+0.33	90	50	+0.7	+0.25	210	1.48	38	1.15	1.31	42.9
37	5.07	11.9	31.7	4.8	29	150	40	+0.31	85	52	+1.9	-/+ 0.55	205	1.05	31	0.74	0.98	36.7
38	5.73	15.1	38.9	2.8	25	120	38	+0.31	105	59	+1.5	-0.6	180	1.1	34	0.88	1.41	29.6
39	6.12	15	39.9	4.2	5 0	145	37	+0.25	115	57	+1.0	+0.35	190	1.29	34	1.01	1.29	27.8

cont. Table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
40	6.26	15.6	40.2	2.9	39	135	39	+0.28	80	58	+1.1	+0.4	175	1.44	28	0.84	1.15	29.5
41	5.96	14.8	39.8	5.8	30	150	22	+0.22	75	60	+1.7	-0.5	230	1.3	25	0.68	0.99	25.8
42	6.15	14.5	40.1	3.8	30	100	35	+0.29	95	60	+0.65	+/- 0.35	200	1.25	33	0.89	1.42	43.2
43	5.39	13.1	36.2	10.7	26	175	42	+0.31	115	62	+1.45	+0.45	260	1.32	**42**	1.15	1.41	54.7
44	4.75	11.1	29.9	12.2	33	115	34	+0.4	90	53	+1.0	-0.35	180	1.02	30	0.86	1.25	49.8
45	5.09	12.8	34.7	1.6	20	150	33	+0.21	75	54	+1.3	-0.5	195	1.41	35	0.77	1.36	25.1
46	3.97	10.5	25.2	6.1	31	135	39	+0.37	85	53	+1.35	+0.35	185	1.21	39	0.9	1.44	32.8
47	5.03	12.4	34	9.7	32	140	29	+0.15	100	57	+0.65	-0.25	200	1.17	29	0.88	1.29	50.5
48	6.16	15.3	42.1	9.9	20	50	31	+0.15	145	59	+1.5	+/-0.5	260	1.51	39	1.15	1.61	48.1
49	4.73	11.6	29.7	4.1	16	150	40	+0.29	85	51	+1.25	+0.45	190	1.45	28	1.1	1.41	29.6
50	6.58	15.5	42.3	4.9	28	130	35	+0.23	95	60	+1.85	+0.7	210	1.21	31	1.01	1.52	50.4
Range	5.5-8.5	12-18	37-51	6-10	200-500	70-120	40	40	120	60	4	1/3 R	150-250	1.5	25-40	1.5	1.75	see: Tilley et al. (2008)

Table 2. Results of hematology, electrocardiography and echocardiography in control dogs.

No of the dog	Results of haematological examination					Results of electrocardiography								Results of echocardiography				
	RBC x 10^{12}	Hb g/dl	Ht %	WBC 10^9/l	PLT 10^9/l	HR	P (ms)	P (mV)	PQ (ms)	QRS (ms)	R (mV)	T (mV)	QT (ms)	LA/ /Ao	FS (%)	RVOT (ms)	LVOT (ms)	LViDd (mm)
1	5.6	6.0	36.5	5.8	288	115	38	+0.22	105	47	+2.2	+0.4	150	1.23	31	0.98	1.26	33.2
2	5.9	6.7	38.2	6.3	321	100	33	+0.29	95	51	+1.5	+0.5	160	1.44	33	0.79	1.08	62.5
3	6.2	5.9	41.2	5.8	401	120	35	+0.14	100	49	+2.8	+0.9	180	1.06	28	1.09	1.37	52.3
4	7.6	7.0	40.5	8.0	412	135	31	+0.19	95	52	+1.0	-0.3	220	1.43	38	0.89	1.18	29.2
5	8.3	7.5	49.3	7.9	324	150	32	+0.28	85	39	+1.8	+0.5	200	1.32	30	1.01	1.35	28.4
6	6.5	7.0	41.8	6.1	333	130	36	+0.32	115	48	+1.8	-0.2	170	1.29	27	0.89	1.13	57.8
7	5.7	6.8	37.9	7.1	367	110	29	+0.19	95	52	+0.9	+0.3	180	1.20	34	1.08	1.53	39.4
8	6.1	6.9	42.1	7.4	224	130	38	+0.28	110	47	+2.2	+0.5	200	1.09	32	0.88	1.22	33.1
9	7.3	7.0	47.2	8.1	407	145	37	+0.22	100	50	+2.5	+0.4	210	1.25	34	0.98	1.67	34.5
10	7.9	7.1	50.1	5.9	365	125	35	+0.21	90	48	+1.2	-0.3	190	1.41	26	0.99	1.39	53.9
11	8.1	8.3	48.2	5.6	382	150	38	+0.19	95	42	+2.0	-0.4	200	1.36	37	0.87	1.62	44.1
12	8.0	5.9	49.5	6.4	412	160	33	+0.27	80	35	+2.7	-0.4	160	1.18	31	0.68	1.11	34.1
13	5.6	7.1	38.6	6.9	399	155	40	+0.37	100	55	+2.1	+0.6	190	1.35	25	0.93	1.28	42.6
14	7.3	7.3	43.8	8.1	355	120	39	+0.23	85	47	+1.2	+0.3	220	1.25	35	0.75	1.09	32.5
15	7.8	8.1	43.0	8.4	312	110	29	+0.15	120	53	+1.7	+0.4	230	1.41	39	1.11	1.47	26.3
16	7.8	6.5	41.6	6.2	405	140	32	+0.29	100	41	+1.1	-0.2	210	1.23	33	0.81	1.21	32.8
17	8.0	6.1	48.1	6.7	453	95	38	+0.31	90	51	+1.1	+0.3	180	1.47	36	0.86	1.39	49.3
18	5.5	5.8	37.7	5.9	393	130	35	+0.27	105	48	+0.9	+0.2	210	1.15	28	0.97	1.59	31.7
19	5.9	7.0	39.5	7.7	410	110	34	+0.32	85	45	+2.1	+0.6	200	1.21	30	1.13	1.35	31.8
20	6.4	6.4	42.7	7.0	444	100	36	+0.20	115	58	+2.0	-0.4	170	1.39	29	1.03	1.44	54.5
Range	5.5-8.5	12-18	37-51	6-10	200-500	90-150	40	40	120	60	4	1/3 R	150-250	1.5	25-40	1.5	1.75	see: Tilley et al. (2008)

Table 3. Relation of CK-MB U/L level to ECG record.

Changes in ECG	Mean CK-MB concentration (range: 4,7-6,3 U/L)	N	SD	Min	Max	Me
0	118.8727	30	62.3784	4.1500	252.1100	110.2500
1	130.9740	15	51.5986	48.7800	211.8500	130.6400
2	250.0580	5	110.2294	138.2200	369.6200	198.3900
Total	135.6216	50	74.6331	4.1500	369.6200	127.8950

H = 7.954225; p = 0.0187 (**)
0 – dogs with normal ECG record
1 – dogs with slight changes in ECG (e.g. increased rhythm, elevated T wave)
2 – dogs with significant changes in ECG (e.g. nodal tachycardia, additional ventricular beats, bradyarrhythmia)
N = number of dogs, SD = standard deviation, Min = minimal value, Max = maximal value, Me = mediana
The statistical analysis showed there to be significant correlations between ECG changes in dogs suffering from babesiosis and the activity of CK-MB in their serum. Significant differences were found between groups "0" and "2" with probability $p<0.05$.

Table 4. Relation of troponin level to changes in ECG record.

Changes in ECG	Mean troponin level (range: <0,2 ng/dl)	N	SD	Min	Max	Me
0	0.66833	12	0.56399	0.200000	1.70000	0.520000
1	1.04833	6	0.43563	0.590000	1.70000	0.960000
2	11.12000	5	10.13938	1.200000	26.70000	9.200000
Total	3.03957	23	6.15484	0.200000	26.70000	0.820000

0 – dogs with normal ECG record
1 – dogs with slight changes in ECG (e.g. elevated rhythm, elevated T wave)
2 – dogs with significant changes in ECG (e.g. nodal tachycardia, additional ventricular beats, bradyarrhythmia)
H = 11.29; p = 0.0035 (**)
N = number of dogs, SD = standard deviation, Min = minimal value, Max = maximal value, Me = mediana
The statistical analysis showed there to be significant correlations between the ECG changes of the dogs suffering from babesiosis and the troponin concentration in their serum. The biggest differences in this regard were noted between groups "0" and "2".

and troponin concentration. An important correlation between the tested parameters was reported in the group of dogs that had significant abnormalities in ECG (e.g. nodular tachycardia, additional ventricular beats, bradyarrhythmia) (Tables 3 and 4).

No correlation was observed between the parameters determined in the haematology test and changes in the ECG of dogs infected with *Babesia* protozoa. The p value for the analysed parameters was $p>0.05$. For dogs in which the shortening fraction was elevated (above 40%), the leukocyte count was statistically significantly higher compared to dogs with a correct shortening fraction ($p=0.04$). Additionally, in all dogs with incorrect shortening fraction, the parameters determined in the echocardiography test were statistically significantly different (higher) than in dogs in which the shortening fraction was correct in the echocardiography test. Statistically significant differences occurred for all tested parameters: LA/Ao ($p=0.013$), FS ($p=0.00001$), RVOT ($p=0.003$) and LVOT ($p=0.0006$). Furthermore, in protozoa-infested dogs with an incorrect shortening fraction, statistical relations in the correlation test were observed only between LA/A0 and RBC, HCT and HGB: r = -0.73, r = -0.73 and r = -0.70, respectively. In these cases, the correlation was negative.

During the study of the relations between ECG results and the echocardiography test results, a correlation between LA/Ao and the T wave amplitude (r = 0.66) was noted in dogs with the incorrect shortening fraction.

Discussion

In recent years, much attention has been paid to cardiac disorders in the course of babesiosis, which can cause the death of the infected animals.

In this study, in the electrocardiography test, the most frequent abnormalities in the ECG record in

dogs with babesiosis were a change in T-wave morphology and an increased sinus rhythm. They were the consequences of hypoxia of the organism which, among other things, subsequently led to the damage of the cardiac muscle (Lobetti 2010).

The observations of Dvir et al. (2004) showed that the frequency of conductivity disturbances in the cardiac muscle in sick dogs presents as follows: changes in the morphology of T wave – 40%, heart-axis deviation – 40%, dilated QRS complex – 32%, atrioventricular block – 7%, premature ventricular beats – 7%. These authors did not report cases of supraventricular and nodular tachycardia, which occurred in one dog in this study. It should also be mentioned that the described studies involved dogs from the Republic of South Africa where the aetiological factor of babesiosis is most commonly *B. rossi* – a more virulent form of *Babesia.* This fact could have had an influence on the obtained results (Matjila et al. 2008, Penzhorn 2011).

It should also be noted that in the course of babesiosis there are many factors which affect the change of the electrocardiogram, e.g. anaemia, hypoxia or uraemia, and the heart muscle damage cannot be determined solely based on ECG result (Lobetti et al. 2012). Nevertheless, in animals with protozoa infection, in which there is an irregular rhythm, the prognosis is poor (Dviret al. 2004).

In this study, in order to confirm heart muscle damage, the serum troponin I (cTnI) level was measured, as well as the creatine kinase isoenzyme (CK-MB) level, which are the markers of myocardium damage (Schoberet al. 1999, Boswood et al. 2009, Lobetti 2012).

Tests of heart muscle damage have become increasingly important in medical and veterinary practice in recent years. Aside from troponin level tests, the method of NT-proNBP determination has also been developed. The increase of troponin concentration level is equivalent to, among other things, the increase of intracardiac pressure, hypoxia of myocardium and increased tension of the sympathetic system (Chetboul et al. 2004, Oyama et al. 2008, Boswood et al. 2009). The elevated NT-proNBP level is a prognostic factor of progress of heart disease (Oyama et al. 2008).

The isoforms I and T are the specific troponin isoforms for the cardiac muscle. They are important and very sensitive markers of cardiac-muscle damage in humans, and hence are used in the diagnostics of myocardial infarction, showing 100% sensitivity and 97% specificity in the diagnosis of this condition (Alpert et al. 2000, Collinson et al. 2000).

It is worth noting that the amino-acid sequence of cardiac troponins in humans and dogs is almost identical (O'Brien et al. 1997). The determination of these protein levels in dogs makes sense when the subject's clinical symptoms persist longer than 8 hours but not longer than 10 days. Studies carried out by Lobetti (2005) showed that an elevated level of troponin persisting for a longer time is an indicator of poor prognosis.

In this study 38% of dogs with babesiosis had a level of troponin I in serum above the limit of the norm (i.e. 0.2 ng/mL), and in 2 dogs that died as a consequence of the disease the limit was significantly exceeded.

Our observations, similarly to the results presented by Lobetti et al. (2012), demonstrated that the troponin I level is not related to the haematocrit level. In 7 dogs in this study, the troponin I level was exceeded and the haematocrit level was normal. On one occasion the haematocrit level was low and troponin I level was normal.

The second marker determined for the assessment of cardiacmuscle damage was the creatine kinase enzyme (CK-MB). After all of the 50 serums obtained from the sick animals had been examined, it appeared that in 49 samples the level of this enzyme in serum was elevated. The studies of Schober et al. (1999) showed that creatine kinase isoenzyme MB is more specific for cardiac muscle damage as compared with creatine kinase, though publications from the last century proved that the assessment of cardiac muscle damage on the basis of troponin I (cTnI) level determination is more reliable than assessment on the basis of determination of CK-MB level or ECG examination (Schoberet al. 1999). This is caused by the fact that elevated CK-MB activity is observed when the liver is damaged, a frequent complication in the course of babesiosis. The specificity of CK-MB level determination in humans for the detection of cardiac muscle damage is significantly higher than in dogs. This is related to the fact that creatine kinase MB isoform in the cardiac muscle in dogs accounts for 4-13% of the total activity of creatine kinase, while in humans it is almost 40%. The other sources of creatine kinase MB isoform include the spleen, muscles, lungs and intestines, which can also be damaged in the course of canine babesiosis (Aktas et al. 1993). It can be concluded that the elevated level of creatine kinase MB isoenzyme may be treated as nonspecific marker of the course of babesiosis in dogs, even in cases with a mild course.

In this study, in the echocardiography test, attention was focused mainly on the assessment of the fractional shortening (FS%) as well as the disturbed speed flow at the outflow from the right (RVOT) and left ventricles (LVOT). Before the examinations started, it had been assumed that, due to anaemia and

decreased haematocrit, changes would occur in cardiac muscle contraction and in blood flow through the heart. The level of the fractional shortening was increased (above 40%) in 12 dogs, constituting 24%. The decreased haematocrit level was also observed in 8 dogs out of these 12, similarly to dogs with babesiosis coming from the RSA (Spotswood et al. 2006). In both studies the dogs with a low haematocrit level had an increased heart rate, a change in morphology of T wave in electrocardiography and an increased fractional shortening. Additionally, in the studies carried out in the RSA, it was noted that the e-point septal separation was substantially reduced in dogs with haematocrit below 20%, while in dogs with haematocrit over 20% the EPSS did not significantly differ from dogs with normal Ht.

The presented results show that cardiac changes are common in canine babesiosis, but most changes are nonspecific and appear to have little clinical significance. Cardiovascular assessment should be based on the assessment of the level of troponin I and CK-MB in the serum of sick animals. High concentrations of these factors might be indicators of poor prognosis.

References

Adaszek Ł, Winiarczyk S, Skrzypczak M (**2009**) The clinical course of babesiosis in 76 dogs infected with protozoa parasites *Babesia canis canis*. Pol J Vet Sci 12: 81-87

Adaszek Ł, Winiarczyk S (**2010**) Application of the SYBR Green real-time HRM PCR technique in the differentiation of the *Babesia canis canis* protozoa isolated in the areas of eastern Poland. Parasitol Res 106: 1253-1256.

Aktas M, Auguste D, Lefebvre HP, Toutain PL, Braun JP (**1993**) Creatine kinase in the dog, a review. Vet Res Commun 17: 353-369.

Alpert JS, Thygesen K, Antman E, Bassand JP (**2000**) Myocardial infarction redefined – a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infraction. J Am Coll Cardiol 36: 950-969.

Boswood A, Dukes-McEwan J, Loureiro J, James RA, Martin M, Stafford-Johnson M, Smith P, Little C, Attree S (**2008**) The diagnostic accuracy of different natriuretic peptides in the investigation of canine cardiac disease. J Small Anim Pract 49: 26-32

Bourdoiseau G (**2006**) Canine babesiosis in France. Vet Parasitol 138: 118-125.

Camacho AT, Pallas E, Gestal JJ, Guitian FJ, Olmeda AS, Goethert HK, Telford SR (**2001**). Infection of dogs in north-west Spain with a *Babesia microti*-like agent. Vet Rec 149: 552-555.

Carret C, Delbecq S, Labesse G, Carcy B, Precigout E, Moubri K, Schetters TP, Gorenflot A (**1999**) Characterization and molecular cloning of an adenosine kinase from *Babesia canis rossi*. Eur J Biochem 265: 1015-1021.

Chetboul V, Tessier-Vetzel D, Escriou C, Tissier R, Carlos C, Boussouf M, Pouchelon JL, Blot S, Derumeaux G (**2004**) Diagnostic potential of natriuretic peptides in the occult phase of golden retriever muscular dystrophy cardiomyopathy. J Vet Intern Med 18: 845-850.

Collinson PO, Premachandram S, Hashemi K (**2000**) Prospective audit of incidence of prognostically important myocardial damage in patients discharged from emergency department. BMJ 320: 1702-1705.

Conrad P, Thomford J, Yamane I, Whiting J, Bosma L, Uno T, Holshuh HJ, Shelly S (**1991**) Hemolytic anemia caused by *Babesia gibsoni* infection in dogs. J Am Vet Med Assoc 199: 601-605.

Costa-Júnior LM, Ribeiro MF, Rembeck K, Rabelo EM, Zahler-Rinder M, Hirzmann J, Pfister K, Passos LM (**2009**) Canine babesiosis caused by *Babesia canis vogeli* in rural areas of the State of Minas Gerais, Brazil and factors associated with its seroprevalence. Res Vet Sci 86: 257-260.

Dvir E, Lobetti RG, Jacobson LS, Pearson J, Becker PJ (**2004**) Electrocardiographic changes and cardiac pathology in canine babesiosis. J Vet Cardiol 6: 15-23.

Heile C, Heydorn AO, Schein E (**2006**) *Dermacentor reticulatus* (Fabricius, 1794)-distribution, biology and vector for *Babesia canis* in Germany. Berl Munch Tierarztl Wochenschr 119: 330-334.

Irwin PJ (**2009**) Canine babesiosis: from molecular taxonomy to control. Parasit Vectors Suppl 1: S4.

Kjemtrup AM, Conrad PA (**2006**) A review of the small canine piroplasms from California: *Babesia conradae* in the literature. Vet Parasitol 138: 112-117.

Lobetti R (**2010**) The pathophysiology of renal and cardiac changes in canine babesiosis: Pathophysiology of canine babesiosis. LAP Lambert Academic Publishing, pp 23-35.

Lobetti RG (**2005**) Cardiac involvement in canine babesiosis. J S Afr Vet Assoc 76: 4-8.

Lobetti R, Kirberger R, Keller N, Kettner F, Dvir E (**2012**) NT-ProBNP and cardiac troponin I in virulent canine babesiosis. Vet Parasitol 190: 333-339.

Matjila PT, Leisewitz AL, Jongejan F, Bertschinger HJ, Penzhorn BL (**2008**) Molecular detection of *Babesia rossi* and *Hepatozoon* sp. in African wild dogs (*Lycaon pictus*) in South Africa. Vet Parasitol 157: 123-127.

Mehlhorn H, Schein E, Voigt WP (**1980**) Light and electron microscopic study on developmental stages of *Babesia canis* within the gut of the tick *Dermacentor reticulatus*. J Parasitol 66: 220-228.

O'Brien PJ, Landt Y, Ladenson JH (**1997**) Differential reactivity of cardiac and skeletal muscle from various species in a cardiac troponin I immunoassay. Clin Chem 43: 2333-2338.

Oyama MA, Fox PR, Rush JE, Rozanski EA, Lesser M (**2008**) Clinical utility of serum N-terminal pro-B-type natriuretic peptide concentration for identifying cardiac disease in dogs and assessing disease severity. J Am Vet Med Assoc 232: 1496-1503.

Penzhorn BL (**2011**) Why is Southern African canine babesiosis so virulent? An evolutionary perspective. Parasit Vectors 4: 51.

Schetters TP, Eling WM (**1999**) Can Babesia infections be used as a model for cerebral malaria? Parasitol Today 15: 492-497.

Schober KE, Kirbach B, Oechtering G (**1999**) Non-invasive assessment of myocardial cell injury in dogs with suspected cardiac contusion. J Vet Cardiol 1: 17-25.

Spotswood TC, Kirberger RM, Koma LM, Thompson PN, Miller DB (**2006**) Changes in echocardiographic variables of left ventricular size and function in a model of canine normovolemic anemia. Vet Radiol Ultrasound, 47: 358-365.

Tilley LP, Smith FWK, Oyama MA, Sleeper MM (**2008**) Manual of canine and feline cardiology. 4th ed., Saunders Elsevier, pp 386-410.

Zahler M, Schein E, Rinder H, Gothe R (**1998**) Characteristic genotypes discriminate between *Babesia canis* isolates of differing vector specificity and pathogenicity to dogs. Parasitol Res 84: 544-548.