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Haematological and biochemical parameters of canine hepatozoonosis in Croatia

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ABSTRACT

Infection with *Hepatozoon canis* has been described recently in 11.8% of Croatian dogs. Hematological and biochemical analyses of 14 apparently asymptomatic, but *Hepatozoon*-positive dogs were performed for the first time in Croatia. Haematological analyses showed severe eosinophilia in 9 cases (64.3%), leukocytosis in 5 (35.7%), neutrophilia in 3 (21.4%), decreased values of HCT and HGB in 2 (14.3%), monocytosis in 3 (21.4%), lymphocytosis in 6 (42.9%), thrombocytopenia in 2 (14.3%) and thrombocytosis in 3 cases (21.4%), where platelet aggregates were detected. Biochemical analyses of dog sera demonstrated highly increased ALP in 7 (50%) and ALT and CK in 2 dogs (14.3% each). AST and GGT were slightly increased in 2 (21.4%) samples. Also, β1-hyperglobulinemia, β1-hypoglobulinemia and γ-hypoglobulinemia were detected in certain samples (28.6%, 21.4% and 92.9%, respectively). Many changes in the biochemical and haematological parameters in this study correlate with previous researches, but some of them show opposite trend. We conclude that clinical signs and laboratory findings in the initial phase of *Hepatozoon* infection, or in the infection of weak intensity, are quite unspecific and similar to those observed in other diseases commonly found in dogs.

Key words: *Hepatozoon*, dogs, haematology, biochemistry

Introduction

Infection with *H. canis* in dogs occurs by ingestion of a tick containing sporulated *H. canis* oocysts. Oocysts rupture and release sporozoites in the dog's intestinal lumen. Sporozoites penetrate the gut wall, invade phagocytic cells and are carried via lymph or blood to the canine parenchymal organs and muscle tissue, where meronts are formed and

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rupture to release merozoites that transform into gamonts in neutrophils and monocytes. The life cycle is completed when the tick feeds on a parasitemic dog and ingests gametocytes; these undergo gametogony and produce oocysts in the tick's body cavity (BANETH et al., 2007).

Up to now two *Hepatozoon* species associated with canine hepatozoonosis have been described: *H. canis* and *H. americanum*. *H. americanum* is the causative agent of canine hepatozoonosis in North America (BANETH et al., 2000), transmitted by *Amblyomma maculatum* ticks (VINCENT-JOHNSON et al., 1997a; BANETH et al., 2003). The agent causes a distinct clinical syndrome in dogs, characterized by fever, lethargy, weight loss, stiffness, signs of pain, paralysis, and ocular discharge (VINCENT-JOHNSON et al., 1997a,b; MacINTIRE et al., 2001). Histopathologically, *H. americanum*-containing lesions can be found in many tissues such as striated muscle, liver, lymph nodes, spleen and in the pancreas (EWING and PANCIERA, 2003). *H. canis* is the cause of Old World canine hepatozoonosis and is transmitted by the brown dog tick, *Rhipicephalus sanguineus* (BANETH et al., 2001; BANETH et al., 2003). The symptomatology of dogs infected with *H. canis* varies from mild or even asymptomatic to severe signs, depending on the parasitemia and the animal's immune state. Pathogenesis of *H. canis* is thought to be weak, because subclinical infections are common, usually causing a milder disease that affects the spleen, lymph nodes, and bone marrow, resulting in anaemia and lethargy (BANETH and WEIGLER, 1997; GAVAZZA et al., 2003). *H. canis* gametocytes can be detected in circulating leukocytes of infected dogs, even in those without clinical signs (VINCENT-JOHNSON et al., 1997b; BANETH et al., 2003).

Hepatozoonosis is often found in association with other infections (GONDIM et al., 1998), especially with other hematozoa commonly observed in dogs, such as *Ehrlichia* and *Babesia* (MUNDIM et al., 2008). On the other hand, *H. canis* can induce a primary clinical disease in dogs (VOYVODA et al., 2004). Existing reports on asymptomatic dogs showed no uniform changes in haematological and biochemical parameters caused by the presence of *H. canis* (PALUDO et al., 2003; ASSARASAKORN et al., 2006; O'DWYER et al., 2006; EIRAS et al., 2007). Therefore, in an attempt to clarify the present situation, we investigated haematological and biochemical parameters of *Hepatozoon*-infected asymptomatic dogs in Croatia and compared obtained results with previously reported ones.

Materials and methods

Blood samples. Blood samples from 14 apparently asymptomatic dogs were collected from different locations throughout Croatia as a part of scientific project activities related to monitoring and control of canine protozoan diseases during the period from 2007 to 2008. Before the venipuncture, all the dogs included in the monitoring had been examined

by a veterinary clinician, according to standard scheme for general clinical investigation (anamnesis, rectal body temperature, visible mucous membranes, quality and rate of the pulse, palpation of the subcutaneous lymph nodes).

Information on age, sex, breed, and other characteristics was gathered using a standardized questionnaire, administered to the owners of each animal (Table 1). Blood samples were stored with EDTA and transported to the laboratory in cold packages. All samples were examined microscopically for *H. canis* gametocytes. Also, all samples were tested for the presence of *Hepatozoon* spp. (PCR), *Ehrlichia* spp. (PCR), *Anaplasma* spp. (PCR), *Babesia* spp. (PCR, ADASZEK and WINIARCZYK, 2008), *Leishmania* spp. (PCR and ELISA, LACHAUD et al., 2002) and *Dirofilaria* spp. (Knott's test). Positive samples were further confirmed by molecular sequencing (VOJTA et al., 2009). Samples were screened for the presence of *Anaplasma phagocytophilum* according to the nested-PCR protocol of MASSUNG et al. (1998). Samples were also screened for the presence of *Ehrlichia* spp. and *Anaplasma* spp., using the primers EHR16SD and EHR16SR that detect all *Ehrlichia* and *Anaplasma* species (INOKUMA et al., 2000) and according to the protocol of MARTIN et al. (2005).

Table 1**.** Origin, age, and breed information on dogs whose haematological and biochemical parameters were determined in the blood

Dog label*	*Hepatozoon* species (isolate group*)	Location (City)	Sex	Age	Breed	Coinfection with
102	*canis* (5)	Rijeka	f	6 months	mongrel	-
105	*canis* (5)	Rijeka	f	6 months	mongrel	-
104	*canis* (6)	Rijeka	f	7 months	mongrel	-
101	*canis* (1)	Rijeka	f	6 months	mongrel	-
103	*canis* (1)	Rijeka	f	2 years	mongrel	-
106	*canis* (4)	Pula	f	14 years	mongrel	-
108	*canis* (5)	Pula	f	1 year	mongrel	-
107	*canis* (6)	Pula	m	1.5 years	mongrel	-
86	*canis* (6)	Slavonski Brod	f	5 years	mongrel	-
87	*canis* (1)	Slavonski Brod	m	5 months	mongrel	-
99	*canis* (1)	Slavonski Brod	m	12.5 years	Istrian cattle driver	*Dirofilaria repens*
100	sp.	Slavonski Brod	f	6 years	Istrian cattle driver	*Dirofilaria repens*
85	*canis* (1)	Slavonski Brod	f	3 years	Istrian cattle driver	-
88	*canis* (1)	Slavonski Brod	m	4.5 months	Istrian cattle driver	-

*data according to our previous results (VOJTA et al., 2009)

Biochemical and haematological analyses. After typing the *Hepatozoon*-positive dogs (methods described in VOJTA et al., 2009), 14 of them were subjected to biochemical and haematological analyses. These 14 dogs were the only *Hepatozoon*-positive dogs (as determined by PCR) whose blood and sera samples were preserved adequately for biochemical and haematological analysis. The blood samples for analysis were collected from the cephalic vein on the day of admission to the Faculty. The samples were stored in sterile tubes with EDTA for haematological analysis and tubes with no anticoagulant, which were centrifuged at 1200 g. The sera obtained were stored at -70 ºC until they were processed. The haematological analyses were performed using an automatic haematology analyzer (Serono 9120, Serono Baker Diagnostic). Differential leukocyte counts were performed on blood films stained by the May-Grünwald-Giemsa method. Biochemical profile was determined according to the standard methods, using an automated biochemistry analyzer (Olympus AU 600, Olympus Diagnostica GMBH) with dedicated reagent kits. The biochemistry panel included the following parameters: urea, creatinine, glucose, inorganic phosphorus, total proteins, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gammaglutamyl transferase (GGT) and creatine kinase (CK). Blood proteins were analyzed by electrophoresis. Electrophoresis was carried out at 200 V for 30 minutes on cellulose acetate strips (Cellogel®, Chemetron, Milan, Italy). A tris-hippurate buffer was used at pH 8.8 (Malta Chemetron, Milan, Italy). The protein fraction relationship was read off a densitometer and the absolute concentration of individual fractions in g/l was calculated from the percentages obtained by a densitometer.

Results

Examination of the sampled animals by a veterinary clinician showed no clinical signs of the disease. Microscope examination revealed no presence of *H. canis* gametocytes in the blood of infected dogs, but all samples were positive to *Hepatozoon*, as confirmed by PCR screening (VOJTA et al., 2009 and Table 1). Sequencing revealed *H. canis* to be the agent involved. Presence of *Ehrlichia* spp., *Anaplasma* spp. and *Babesia* spp. was excluded from all samples by PCR and infection by *Leishmania* spp. by PCR and ELISA. *Dirofilaria* spp. was found in 2 dogs (dog labels 99 and 100) using Knott's test and determined on a morphological basis as *D. repens*.

Fourteen blood samples of *Hepatozoon*-positive dogs (as tested by PCR) were haematologically and biochemically analysed. Results of haematological examinations, serum biochemical analyses and serum protein electrophoresis are presented in Table 2. Haematological analyses showed abnormalities such as: eosinophilia in 9 cases (64.3%), leukocytosis in 5 (35.7%), neutrophilia in 3 (21.4%), decreased values of HCT and HGB in 2 (14.3%), monocytosis in 3 (21.4%), lymphocytosis in 6 (42.9%), thrombocytopenia in 2 (14.3%) and thrombocytosis in 3 cases (21.4%), where platelate aggregates were

Table 2. Hematological and biochemical parameters measured from the blood of 14 dogs positive to *Hepatozoon*

	Dog label															
Parameter	102	105	104	101	103	106	108	107	86	87	99	100	85	88	Units	Reference
RBC	5.21	6.02	4.67	6.03	7.67	6.08	6.97	7.5	7.06	5.58	5.56	6.75	7.72	6.27	10^{12}/L	5.4 - 7.8
HGB	111	134	107	145	186	151	172	180	171	129	140	164	179	158	g/L	130-190
HCT	0.334	0.407	0.312	0.418	0.562	0.439	0.502	0.521	0.505	0.377	0.39	0.481	0.532	0.457	L/L	0.37 - 0.54
MCV	64.2	67.6	66.9	69.3	73.3	72.2	72	69.5	71.5	67.5	70.1	71.2	68.9	72.9	fL	64 - 74
MCH	21.3	22.3	22.9	24	24.3	24.8	24.7	24	24.2	23.1	25.2	24.3	23.2	25.2	pg	22 - 27
MCHC	332	329	343	347	331	344	343	345	339	342	359	341	336	346	g/L	340 - 360
RDW	17.9	15.2	18.1	14.9	14.2	14.6	14.8	15.2	16.2	16	14.9	15.7	15.7	15.5	%	12 - 15
PLT	532	526	512	154	184	336	208	59	292	291	273	401	303	391	10^9/L	160 - 430
MPV	12.2	11.8	11.7	11.9	10.8	11	11.7	11.9	10.7	12.1	12.7	9.3	11.7	11.4	fL	6.7 - 11.1
WBC	25.8	23.8	23.6	16	14.3	15.1	14.9	17	17.4	17.6	14.9	21.3	10.4	16	10^9/L	6 - 17
SG	12.9	9.52	10.38	7.52	7.72	8.15	8.79	9.69	14.79	11.26	8.05	12.99	5.51	8.16	10^9/L	3 - 11.5
NSG	0.52	0.95	0	0	0	0	0	0	0.17	0	0	0	0.1	0	10^9/L	0 - 0.3
LIM	4.39	7.85	5.19	8.32	2	4.38	4.32	3.06	1.39	3.17	5.66	7.88	3.74	5.28	10^9/L	1 - 4.8
MO	1.55	0.95	1.89	0	0.29	0	0	0.68	0.35	1.41	0	0	0.42	0	10^9/L	0.15 - 1.35
EO	6.45	4.52	6.14	0.16	4.29	2.57	1.79	3.57	0.7	1.7	1.04	0.43	0.62	2.56	10^9/L	0.1 - 1.25
BAS	0	0	0	0	0	0	0	0	0	0	0.15	0	0	0	10^9/L	<0.1
EBL	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
UREA	1.6	3.4	4.1	7.1	5.8	5.2	5.4	4.4	6.7	3.3	2.8	4.2	8.6	7.3	mmol/L	2.5 - 8.9
CREA	49	58	51	76	88	68	95	85	48	49	66	65	58	73	µmol/L	44 - 124
TP	54	52	51	64	72	75	64	69	72	58	67	73	71	74	g/L	54 - 71*
ALB	25.49	22.57	24.53	30.21	30.89	30.22	29.95	30.5	30.82	25.52	27.6	32.85	35.29	29.38	g/L	26 - 33*
α1	4	2.76	3.57	3.9	3.24	6.15	3.26	2.97	4.32	3.48	3.95	3.36	2.48	3.03	g/L	2.0 - 5.0*
α2	4.16	3.59	5.1	6.08	5.4	7.58	3.33	3.73	6.91	5.45	6.03	5.77	7.67	4.88	g/L	3.0 - 11.0*
β1	6.32	8.42	4.95	5.5	15.48	13.05	22.08	10.63	10.73	7.08	10.99	24.6	7.53	14.95	g/L	7.0 - 13.0*
β2	9.88	9.98	8.47	9.79	9.79	10.88		11.32	12.82	9.86	11.46		12.14	12.95	g/L	6.0 - 14.0*
γ	4.16	4.68	4.39	8.51	7.2	7.12	5.38	9.87	6.41	6.61	6.97	6.42	5.89	8.81	g/L	9.0 - 22.0*
GLUC	4.9	5.4	6.2	5.3	2.4	4.1	5	4.4	5.9	6	5.5	4.5	4.9	4.7	mmol/L	4.3 - 6.7
AST	59	46	54	37	46	66	44	40	27	24	18	38	23	28	IU/L	16 - 43
ALT	39	31	40	25	37	435	164	41	32	26	34	60	62	55	IU/L	15 - 58
GGT	2	2	1	1	1	8	9	4	3	2	1	1	8	4	IU/L	1 - 5
ALP	223	86	133	101	41	590	55	39	57	101	48	70	124	89	IU/L	10 - 73
CK	875	260	671	246	124	72	113	114	122	228	133	210	89	119	IU/L	40 - 254
P	3.1	2.4	2.9	2.5	1.3	1.5	2	1.2	1	2.4	1.7	2	1.7	1.9	mmol/L	0.8 - 2.0

RBC – red blood cells, HGB – haemoglobin, HCT – hematocrit, MCV – mean cell volume, MCH – mean corpuscular hemoglobin, MCHC - mean corpuscular hemoglobin concentration, RDW – red blood cell distribution width, PLT – platelets, MPV – mean platelet volume, WBC – white blood cells, SG – segmented neutrophils, NSG – band neutrophils, LIM – lymphocytes, MO – monocytes, EO – eosinophils, BAS – basophils, EBL – erythroblast, CREA – creatinine, TP – total protein, ALB – albumin, α1, α2, β1, β2, γ –globulins, GLUC – glucose, AST - aspartate aminotransferase, ALT – alanine aminotransferase, GGT – gammaglutamyl transferase, ALP – alkaline phosphatase, CK – creatinine kinase, P – inorganic phosphorus. Referent data from MEYER and HARVEY (2004); * from KANEKO et al. (1997).

Table 3. Statistical analysis of haematological and biochemical parameters measured from the blood of 14 dogs positive to *Hepatozoon*

Parameter	Units	Reference	Mean	SD	Min	Max
RBC	10^{12}/L	5.4 - 7.8	6.36	0.95	4.67	7.72
HGB	g/L	130 - 190	151.93	25.36	107	186
HCT	L/L	0.37 - 0.54	0.45	0.08	0.31	0.56
MCV	fL	64 - 74	69.79	2.61	64.2	73.3
MCH	pg	22 - 27	23.82	1.13	21.3	25.2
MCHC	g/L	340 - 360	341.21	7.71	329	359
RDW	%	12 - 15	15.64	1.14	14.2	18.1
PLT	10^9/L	160 - 430	318.71	143.29	59	532
MPV	fL	6.7 - 11.1	11.49	0.83	9.3	12.7
WBC	10^9/L	6 - 17	17.72	4.33	10.4	25.8
SG	10^9/L	3 - 11.5	9.67	2.55	5.51	14.79
NSG	10^9/L	0 - 0.3	0.12	0.28	0	0.95
LIM	10^9/L	1 - 4.8	4.76	2.13	1.39	8.32
MO	10^9/L	0.15 - 1.35	0.54	0.66	0	1.89
EO	10^9/L	0.1 - 1.25	**2.61**	**2.09**	0.16	**6.45**
BAS	10^9/L	<0.1	0.01	0.04	0	0.15
EBL			0.07	0.27	0	1
UREA	mmol/L	2.5 - 8.9	4.99	1.96	1.6	8.6
CREA	µmol/L	44 - 124	66.36	15.43	48	95
TP	g/L	54 - 71*	65.43	8.47	51	75
ALB	g/L	26 - 33*	28.99	3.46	22.57	35.29
α1	g/L	2.0 - 5.0*	3.61	0.89	2.48	6.15
α2	g/L	3.0 - 11.0*	5.41	1.40	3.33	7.67
β1	g/L	7.0 - 13.0*	11.59	5.99	4.95	24.60
β2	g/L	6.0 - 14.0*	10.78	1.38	8.47	12.95
γ	g/L	9.0 - 22.0*	6.60	1.68	4.16	9.87
GLUC	mmol/L	4.3 - 6.7	4.94	0.96	2.4	6.2
AST	IU/L	16 - 43	39.29	14.33	18	66
ALT	IU/L	15 -58	77.21	108.75	25	**435**
GGT	IU/L	1 - 5	3.36	2.90	1	9
ALP	IU/L	10 -73	125.5	**142.18**	**39**	**590**
CK	IU/L	40 - 254	241.14	236.47	72	**875**
P	mmol/L	0.8 - 2.0	1.97	0.63	1	**3.1**

RBC - red blood cells, HGB - haemoglobin, HCT - haematocrit, MCV - mean cell volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentration, RDW -- red blood cell distribution width, PLT - platelets, MPV - mean platelet volume, WBC - white blood cells, SG - segmented neutrophils, NSG - band neutrophils, LIM - lymphocytes, MO - monocytes, EO - eosinophils, BAS - basophils, EBL - erythroblast, CREA - creatinine, TP - total protein, ALB - albumin, α1, α2, β1, β2, γ - globulins, GLUC - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase, GGT - gammaglutamyl transferase, ALP - alkaline phosphatase, CK - creatinine kinase, P - inorganic phosphorus. Referent data from MEYER and HARVEY (2004); * from KANEKO et al. (1997). Values that are significantly outside referent values are bolded.

detected. Biochemical analyses of dog sera demonstrated highly increased ALP in 7 (50%) and ALT and CK in 2 dogs (14.3% each). AST and GGT were slightly increased in 2 (21.4%) samples. Although the total protein, albumin and α1-, α2- and β2-globulin values were within the normal range in all tested samples, serum protein electrophoresis revealed β1-hyperglobulinemia, but also β1-hypoglobulinemia in certain samples (28.6% and 21.4%, respectively). Hypogammaglobulinemia was the only consistent finding in this study, found in 13 out of 14 dogs (92.9%).

From 14 analysed blood samples, three of them (dog labels 102, 104 and 105) showed significant discrepancy in most of the tested parameters compared with the reference range, indicating the biochemical and hematological disbalance that could be caused by *Hepatozoon* invasion (Table 2). Among changes in other parameters, PLT, WBC, Eo, ALP and CK concentrations were significantly increased for these dogs. It is interesting to notice that these 3 dogs originated from the same location. Furthermore, two other dogs (labeled 106 and 108) had highly increased ALT, GGT and ALP values. Dog 106 had ALP as high as 8 times above the upper limit of the referent range. Many other haematological and biochemical parameters of other tested dogs were affected in some way, but not with high significance.

Statistical analysis showed that, when considering the blood parameters of all tested dogs, only Eo and ALP values significantly outranged the reference values, while ALT and CK levels were elevated only for some animals (Table 3). The mean values of the rest of the haematological and biochemical parameters were inside the referent range (Table 3).

According to our previous work (VOJTA et al., 2009), Croatian *H. canis* isolates were divided into 6 groups, on the basis of the individual point mutations. This research showed no connection between the isolate group and haematological and biochemical parameters of tested *Hepatozoon*-positive dogs (Tables 1 and 2). Among these dogs, blood parameters of the dog infected with *Hepatozoon sp.* were tested as well (dog label 100), and they were generally the same as for the dogs infected by *H. canis* (Table 2).

Discussion

Canine diseases caused by tick-borne pathogens may be considered among the most significant groups of emerging diseases worldwide. Hepatozoonosis is of particular concern among these infections because it has a long incubation period or may exist in subclinical form. Canine hepatozoonosis due to *H. canis* is generally not associated with clinical signs. Some investigators have considered *H. canis* to be non-pathogenic and, when signs of the disease were evident in infected dogs, they were attributed to other causes such as dirofilariasis, distemper, generalized demodicosis, or leishmaniasis (GONDIM et al., 1998; GAVAZZA et al., 2003). Although it is widely considered non-pathogenic, *H. canis* can be a primary pathogen in dogs, causing depression, anorexia/dysorexia and weight

loss, as well as fever and lymphadenomegaly, or, in the most serious cases, pale mucous membranes and muscular pain (BANETH et al., 2007; MUNDIM et al., 2008). The dogs tested in this study were apparently healthy. *H. canis* was previously detected in asymptomatic dogs (VOJTA et al., 2009).

The haematological and biochemical analyses of 14 dogs showed alterations in many parameters. Clear signs of eosinophilia in most of the 14 tested dogs might indicate parasitic infection. It is also possible that the elevated number of eosinophils results from the eosinophilic myositis. A prevalence of eosinophilia in canine hepatozoonosis was described for the first time by GAVAZZA et al., 2003. Also, elevated WBC number, leukocytosis, neutrophilia and monocytosis in some dogs are in concordance with previous reports (BANETH et al., 1995; BANETH and WEIGLER, 1997; GONDIM et al., 1998; GAVAZZA et al., 2003; VOYVODA et al., 2004; ASSARASAKORN et al., 2006).

In contrast to the work of the authors mentioned above, no clear signs of anaemia (except for two dogs with slightly decreased RBC, HGB and HCT values) or lymphopenia were observed in our study. Interestingly, we detected thrombocytopenia in one dog (as described by BANETH et al., 1995; GAVAZZA et al., 2003; VOYVODA et al., 2004), but also thrombocytosis in our *Hepatozoon*-positive dogs. Thrombocitosis in *H. canis* infection has not been reported before. Results of serum biochemical values showed a similar trend in all cases and included alterations in many parameters. Serum protein electrophoresis of our samples showed an increase or some slight decrease in the β1-globulin fraction, while α1-, α2- and β2-globulin concentrations remained within the reference range. This finding contrasts with the results of GAVAZZA et al. (2003) where a general increase in β- and decrease in the α-globulin range was found. Also, γ-hyperglobulinemia was less frequently detected in that study. On the other hand, we observed the opposite trend (γ-hypoglobulinemia) in 13 out of 14 samples investigated. It was assumed by GAVAZZA et al. (2003) that the involvement of globulins may represent a response to the persistent inflammatory changes associated with the tissue stages of the parasite. Immunoglobulin deficiency and transient γ-hypoglobulinemia also occur in dogs. Dogs with selective IgA deficiency have been recognized in several breeds, including German Shepherd dogs. Clinically significant decreases in either IgA or IgM have been reported in the Shar-Pei breed, with decreased lymphocyte response to mitogens (RIVAS et al., 1995; WERNER and TURNWALD, 1999). IgG deficiency has been demonstrated in Weimeraners, in individuals with recurrent bacterial infections or infections refractory to treatment (DAY et al., 1997). On the other hand, an increase of β-globulin fractions may be due to polyclonal lymphatic activation (BANETH et al., 1995).

Other biochemical abnormalities in serum included an increase in the values of ALP, AST, GGT, ALT and CK in some samples, but no TP increase or a decrease in the albumin concentration were observed (in contrast to BANETH et al., 1995; GAVAZZA et al., 2003). Elevation of ALP and CK levels was observed in all previous studies (BANETH et al.,

1995; BANETH and WEIGLER, 1997; GONDIM et al., 1998; BANETH et al., 2001; GAVAZZA et al., 2003; ASSARASAKORN et al., 2006; EIRAS et al., 2007). The frequently observed very high CK levels may be explained by muscular damage associated with hepatozoonosis (BANETH et al., 1995). Elevated levels of ALP may be related to the chronic disease. Furthermore, elevated alkaline phosphatase activity could result from higher osteoblastic activity or liver necrosis (KRAFT and DURR, 1995; RICH and COLES, 1995), or, even more likely, from cholestasis.

Elevated CK indicates myositis, which is the most common clinical sign of hepatozoonosis described in experimental invasions of dogs (BANETH et al., 1995; MacINTIRE et al., 1997; PANCIERA et al., 2000; PANCIERA and EWING, 2003; EVANS et al., 2004). In our study 8 (57.1%) cases of ALP and just 2 (14.3%) cases of significant CK elevation were observed. Elevated levels of liver enzymes AST and ALT, observed in our dogs and also by other researches, indicate changes in liver function that are probably connected to the life-cycle of *Hepatozoon*, whose merogony takes place in the spleen, liver, bone marrow, periosteum and probably some other organs (BANETH et al., 2007).

Inconsistent changes in some haematological and biochemical parameters (e.g. PLT, β1-globulins, see Table 2) and significant parameter changes in just a few cases (dogs 102, 104, 105, 106, 108) might result from chronic infection with *Hepatozoon* (or/and some other parasite). As mentioned, all dogs tested in this study were apparently healthy. Therefore, we conclude that sub-clinical *Hepatozoon* infection, with undetectable blood gamonts, cannot be confirmed biochemically and haematologically, because these signs are rather unspecific.

Conclusions

Since we studied apparently asymptomatic dogs, the general lack of severity of investigated parameter changes may be explained by a low degree of infection and the fact that *H. canis* infection is often sub-clinical or may cause a mild disease. Haematological and biochemical analyses of asymptomatic, but *Hepatozoon*-positive dogs in this study showed quite random and insignificant changes in the clinical picture that were hard to interpret. Many changes in biochemical and haematological parameters correlate with previous researches, but some of them show opposite trends. Also, the results of previous studies are not thoroughly consistent. It may be concluded here that clinical signs and laboratory findings in canine hepatozoonosis are quite unspecific and similar to those observed in other diseases commonly found in dogs. Therefore, microscopic examination of blood samples and detection and typization of *Hepatozoon* species by molecular methods are a prerequisite for confident diagnosis of this disease. Moreover, beside the application of indispensible molecular biology methods, collection of more clinical data from naturally infected dogs would be crucial to understand pathogenicity of *H. canis*.

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SAŽETAK

Nedavno je utvrđeno da je 11,8% hrvatskih pasa invadirano vrstom *Hepatozooon canis*. Analize hematoloških i biokemijskih pokazatelja 14 naizgled zdravih, ali na Hepatozoon-pozitivnih pasa napravljene su prvi put u Hrvatskoj. Hematološke analize pokazale su izrazitu eozinofiliju u devet slučajeva (64,3%), leukocitozu u pet (35,7%), neutrofiliju u tri (21,4%), smanjene vrijednosti HCT i HGB u dva (14,3%), monocitozu u tri (21,4%), limfocitozu u šest (42,9%), trombocitopeniju u dva (14,3%) i trombocitozu u tri slučaja (21,4%), kod kojih su bili uočeni agregati trombocita. Biokemijske analize seruma pokazale su visoko povišenu razinu ALP u sedam (50%) te ALT i CK u dva psa (14,3% svaka), a AST i GGT vrijednosti bile su lagano povišene u dva uzorka (21,4%). Također, u određenim uzorcima utvrđene su β1-hiperglobulinemija, β1-hipoglobulinemija i γ-hipoglobulinemija (redom 28,6%, 21,4% i 92,9%). Mnoge od opaženih promjena biokemijskih i hematoloških pokazatelja u skladu su s prethodnim istraživanjima, no neke od njih pokazuju suprotan trend. Zaključili smo da su klinički znakovi i laboratorijski nalazi u početnoj fazi infekcije vrstama *Hepatozoon*, kao i kod infekcija slabog intenziteta, prilično nespecifični i slični onima opaženima kod drugih bolesti koje često pogađaju populaciju pasa.

Ključne riječi: hepatozoon, psi, hematologija, biokemija