DE GRUYTER OPEN

DOI: 10.1515/fv-2017-0017

FOLIA VETERINARIA, 61, 2: 44—49, 2017

HAEMATOPATHOLOGICAL CHANGES IN DOGS AFFECTED WITH *EHRLICHIA CANIS* IN LESVOS

Geromichalou, A., Faixová, Z.

Institute of pathological physiology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
Slovakia

zita.faixova@uvlf.sk

ABSTRACT

Canine Ehrlichiosis is an important immunosuppressive tick borne disease in dogs. The geographical distribution and transmission is mostly related with *Rhipicephalus sanguineus* which acts as a vector. There is no predilection of age or sex; all breeds may be infected with Canine Monocytic Ehrlichiosis (CME). The primary targets are monocytic cells. Platelet disorders and serum protein alterations are the principal hematological and biochemical consequences of infections. Clinical signs are almost non-specific. A definitive diagnosis requires: visualization of morulae within monocytes on cytology, detection of serum antibodies with *E. canis,* the IFA test, or the PCR. The objective of this study was to present information about haematological and biochemical tests of *E. canis* infected dogs in Lesvos island in Greece, which is an endemic area.

Key words: dog; *Ehrlichia canis*; hematology; Lesvos island

INTRODUCTION

The species *Ehrlichia canis,* a gram negative obligate intracellular bacterium of the genus *Ehrlichia* (family *Anaplasmataceae),* is the primary cause of Canine Monocytic Ehrlichiosis (CME). It is transmitted by the brown dog tick *Rhipicephalus sanguineus* [1]. The disease was first described in Algeria in 1935 and in southern Africa in 1938. It has a worldwide distribution, apart from Australia and New Zealand, although it is more prevalent in subtropical and tropical areas [15]. A wide variety of clinical and haematological signs and 3 phases have been recognized: acute, subclinical and chronic. The non-specific clinical signs of the acute disease are: high fever, depression, lethargy, and anorexia. The physical examination reveals: lymphadenomegaly, splenomegaly and haemorrhagic tendency with dermal petechiae, ecchymoses, and epistaxis. Thrombocytopenia is the most common hematological finding. Non-regenerative anaemia and a decline in leukocyte count may also occur during this phase. During the subclinical phase, which is characterised by persistent rickettsemia, there are no overt clinical signs and the haematological parameters

usually fall into the normal range, although the platelet count may be in the lower normal range. The chronic form is characterised by pancytopenia due to suppression or destruction of the bone marrow. In this stage, dogs may succumb to fatal secondary bacterial infection and/or bleeding [4, 8, 10].

This research was focused on Ehrlichiosis in dogs in the area of Lesvos, a Greek island in North East Aegean Sea. The vegetation and the climate appears to be favourable for ticks and other ectoparasites. The results coming from dogs in different local areas of this island will be presented, since in endemic areas like Greece, it is a major cause of persistent and life threatening thrombocytopenia and a large number of stray and domestic dogs get infected by *Ehrlichia canis* every year in Lesvos.

MATERIALS AND METHODS

Area and investigated material

Lesvos is the 3rd biggest Greek island situated in the North East Aegean Sea. It has 1,633 km^2 and a population of 100,000 habitants. The climate consists of dry hot summers and short cool winters. The air temperature has an annual average of 18 °C, 10 °C from January — February, and 36 °C in July-August. Rainfall occur during October — March. The vegetation consists of olive trees, pine forests and low bushes and grass. Agricultural activities with many small goat and sheep farms, but also many stray dogs and cats make *Ehrlichia Canis* a very common disease. The selection of 24 dogs, 12 domestic and 12 stray, was made in equal number, 8 dogs (4 domestic and 4 stray) from 3 different areas of the island, A, B, and C. (Fig. 1). Different breeds, sex and ages were tested at a local private clinic.

Methods

A random selection of 24 dogs was made in Lesvos island and these dogs were brought to a private clinic for examination. History, clinical examination, blood collection and rapid Speed Ehrli tests were made. By this test the positive infected were distinguished from the negative dogs. Blood samples were analysed and the results evaluated, so a more definite haematological and biochemical presentation of *E. canis* in Lesvos was demonstrated.

Blood sampling

Blood samples were collected by aspiration from jugular or cephalic veins using a 24 gauge needle and a 0.2 ml syringe, under aseptic conditions. The blood was transferred into tubes with EDTA to prevent clotting and into two tubes containing no anticoagulants. The unclotted sample was used for the determination of haematological parameters employing an Exigo veterinary haematology analyser.

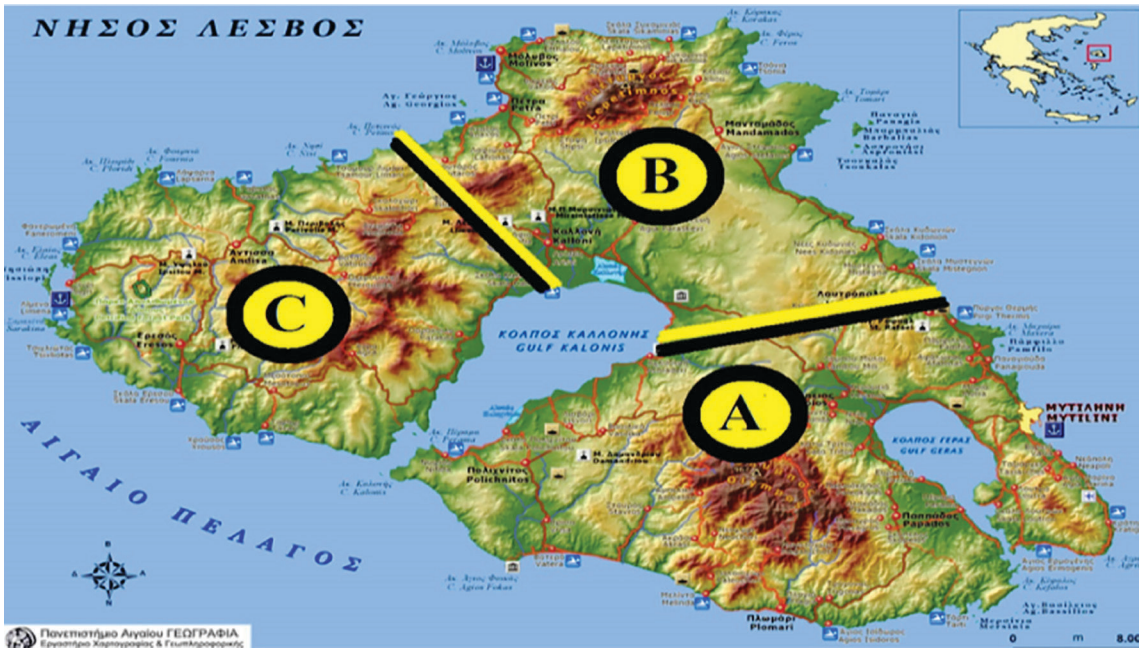

Fig. 1. Map of Lesvos island with areas A, B, C

The two tubes were centrifuged to separate serum from the blood. One tube was used for the determination of biochemical parameters using spot chemII. The other tube was refrigerated for further use.

Speed Ehrli Test

Speed Ehrli is a highly sensitive test which ensures diagnosis before the disease becomes chronic. It is based on the detection of anti *Ehrlichia canis* antibodies and it is a membrane immunochromatography method. Whole blood with or without an anticoagulant, serum or plasma can be used. It has reliability compared with a reference technique, indirect immunofluorescence (IFAT) shows 87 % sensitivity and 95 % specificity. The equipment consists of one test device, one single use pipette and one bottle of reagent. One drop of the sample is pipetted into a well when using an anticoagulant. When using whole blood without anticoagulant, 2 drops of the sample are transferred directly into a well from a syringe. Then 5 drops of the reagent are added and the reading is made after 15 minutes of incubation. The result appears in a result window. The negative test is one single pink band in the right hand side of the window. A positive test is 2 pink bands in the window. After the treatment, the antibodies falls slowly. The result that remains positive may be due to re-infestation. Seroconversion is slow (4 weeks), so if the results are negative but clinical signs show infection, the test should be repeated 15 days later. For this study, blood sample with anticoagulant was used.

RESULTS

From the 24 dogs examined with Speed Ehrli test, 58.33 % were found positive (Patient No. 1 — Patient No. 14), while 41.67 % were negative (Patient No. 15 — Patient No. 24). The hematological parameters of positive dogs are presented in Table 1.

The levels of biochemical parameters in positive dogs are presented in Table 2.

The distribution of positive dogs in areas A, B, C is presented in Table 3. From the 24 examined dogs, 14 were found positive.

The haematological parameters of negative dogs are presented in Table 4.

The biochemical parameters of negative dogs are presented in Table 5.

DISCUSSION

From the 24 randomly chosen dogs examined with Speed Ehrli test in Lesvos from October 2015 until October 2016, 58.33 % were found positive. The remaining dogs (41.67 %) were found negative and their haematological profile showed no signs of anaemia or thrombocytopenia. Patents No. 1 — No. 14 were positive when tested by speed Ehrli test. More precisely, in area A, 50 % of the dogs were positive and from them 25 % were domestic and 75 % were stray dogs. In the area B, 62.5 % of animals were positive and from them 60 % were domestic and 40 % stray dogs. In the area C, 62.5 % of the dogs were positive, of them, 60 % were domestic and 40 % stray dogs. In area A, where the capital town is situated, there was a slightly lower prevalence of infected dogs, while in the areas B and C with low vegetation and many agricultural activities and small farms, the prevalence of infection was higher.

Most of the dogs found infected had clinical signs such as: petechiae, ecchymoses, epistaxis, anorexia, lethargy, weight loss, pale mucous membranes, and oculonasal discharges [2]. Ticks and other ectoparasites were found on many of them, maybe due to insufficient protective measures taken by their owners, such as collar and spot on, or due to a total absence of protection from the stray dogs which moved around quite freely. Even domestic dogs, because of the mild climate, spend most of their time outside households, in gardens, fields and had access to neighbouring small individual farms with sheep and goats,.

In area A there was an increased number of stray dogs, in addition to domestic dogs. Thrombocytopenia is considered to be the most common haematological abnormality in dogs infected with *E. canis* [6, 7, 9, 14]. In our study, platelet (PLT) values below 200 x $10^9.l^{-1}$ were found in 100 % of the dogs which indicated thrombocytopenia. Among them, 35.71 % had PLT ranges lower than $150 \times 10^9.l^{-1}$ and 64.29 % had platelet ranges between $151—199 \times 10^9.l^{-1}$. Many of them had petechiae, ecchymoses, epistaxis and retina haemorrhages as clinical signs (9 of 14 infected dogs). Anaemia is also a common pathological clinical abnormality related to *E. canis* infection [10, 12]. In this study, haemoglobin was found below the normal range in 57.14 % of the infected dogs. A low haemoglobin concentration is a sign of anaemia. The haemoglobin ranges of 28.57 of dogs were above normal and 14.29 % had normal ranges, maybe due to increased haematopoiesis or

Table 1. Percentage of dogs with positive haematological values

Parameter	Reference range	Below [%]	Within [%]	Above [%]
WBC [$10^9.l^{-1}$]	6.0—17.0	–	100	–
LYM [$10^9.l^{-1}$]	0.9—5.0	–	100	–
MID [$10^9.l^{-1}$]	0.3—1.5	–	92.86	7.14
GRAN [$10^9.l^{-1}$]	3.5—2.0	–	92.86	7.14
HGB [$g.dl^{-1}$]	12—18	57.14	14.29	28.57
HCT [%]	37—55	64.29	35.71	–
RBC [$1012.l^{-1}$]	5.5—8.5	64.29	35.71	–
MCV [fl]	60—72	57.14	35.71	7.14
MCH [pg]	19.5—22.5	35.72	7.14	57.14
MCHC [$g.dl^{-1}$)	32—38.5	42.86	50	7.14
RDW [%]	12—17.5	28.58	21.42	50
RDWa [fl]	35—53	21.43	57.14	21.43
PLT [$10^9.l^{-1}$]	200—500	100	–	–
MPV [$10^9.l^{-1}$]	5.5—10.5	–	85.72	14.28
LYM [%]	0.0—99.9	–	100	–
MID [%]	0.0—99.9	–	100	–
GRAN [%]	0.0—99.9	–	100	–

WBC — white blood cells; LYM — lymphocyte; MID — rare cells and white blood cell precursors; GRAN — granulocyte; HGB — haemoglobin; HCT — haematocrit; (packed cell volume); RBC — red blood cell; MCV — mean corpuscular volume; MCH — mean corpuscular haemoglobin; MCHC — mean corpuscular haemoglobin concentration; RDW — red cell distribution width expressed in %; RDWa — red cell distribution width expressed in fl; PLT — platelet; MPV — mean platelet volume

Table 2. Percentage of dogs with positive biochemical values

Parameter	Reference range	Below [%]	Within [%]	Above [%]
BUN [$mg.dl^{-1}$]	6—33	7.14	78.57	14.29
GLU [$mg.dl^{-1}$]	75—117	14.29	71.42	14.29
ALP [$IU.l^{-1}$]	< 132	–	92.86	7.14
TP [$g.dl^{-1}$]	5.3—7.9	7.14	78.57	14.29
ALT [$IU.l^{-1}$]	< 123	–	92.86	7.14
CRE [$mg.d^{-1}$]	0.6—1.6	7.14	85.72	7.14

BUN — blood urea nitrogen; GLU — glucose; ALP — alkaline phosphatase TP — total protein; ALT — alanine transferase; CR — creatinine

Table 3. Positive dogs found in areas A, B, C

	Area A	Area B	Area C	Total
Stray	3	2	2	7
Domestic	1	3	3	7
Total	**4**	**5**	**5**	**14**

Tab. 4. Percentage of dogs with negative haematological values

Parameter	Reference range	Below [%]	Within [%]	Above [%]
WBC [$10^9.l^{-1}$]	6.0 —17.0	–	100	–
LYM [$10^9.l^{-1}$]	0.9—5.0	–	100	–
MID [$10^9.l^{-1}$]	0.3—1.5	–	100	–
GRAN [$10^9.l^{-1}$]	3.5—12.0	–	100	–
HGB [$g.dl^{-1}$]	12—18	–	100	–
HCT [%]	37—55	10	90	–
RBC [$10^{12}.l^{-1}$]	5.5–8.5	–	100	–
MCV [fl]	60–72	10	90	–
MCH [pg]	19.5—22.5	–	10	90
MCHC [$g.d^{-1}$]	32.3—8.5	–	100	–
RDW [%]	12—17.5	–	40	60
RDWa [fl]	35—53	–	70	30
PLT [$10^9.l^{-1}$]	200–500	–	100	–
MPV [$10^9.l^{-1}$]	5.5—10.5	10	90	–
LYM [%]	0.0—99.9	–	100	–
MID [%]	0.0—99.9	–	100	–
GRAN [%]	0.0—99.9	–	100	–

WBC — white blood cells; LYM — lymphocyte; MID – rare cells and white blood cell precursors; GRAN — granulocyte; HGB — haemoglobin; HCT — haematocrit; (packed cell volume); RBC — red blood cell; MCV — mean corpuscular volume; MCH — mean corpuscular haemoglobin; MCHC — mean corpuscular haemoglobin concentration; RDW — red cell distribution width expressed in %; RDWa — red cell distribution width expressed in fl; PLT — platelet; MPV — mean platelet volume

Table 5. Percentage of dogs with negative biochemical values

Parameter	Reference range	Below [%]	Within [%]	Above [%]
BUN [$mg.dl^{-1}$]	6—33	10	90	–
GLU [$mg.dl^{-1}$]	75—117	–	100	–
ALP [$IU.l^{-1}$]	< 132	–	100	–
TP [$g.dl^{-1}$]	5.3—7.9	40	60	–
ALT [$IU.l^{-1}$]	< 123	–	100	–
CRE [$mg.d^{-1}$]	0.6—1.6	–	100	–

BUN — blood urea nitrogen; GLU — glucose; ALP — alkaline phosphatase; TP — total protein; ALT — alanine transferase; CRE — creatinine

due to a different stage of the disease. The haematocrit was found below the normal range in 64.29 % of dogs which is also an indication of anaemia, while 35.71 % of the dogs had normal haematocrit maybe because they were at the subclinical phase of infection when no anaemia is usually detected. The red blood cells were below the normal range in 64.28 % of the dogs indicating anaemia. Normal values were determined in 35.72 % of the dogs indicating probably a subclinical phase of the disease.

The mean corpuscular volume (MCV) was below the normal range in 57.14 % of the dogs suggesting microcytic anaemia and 35.72 % showed normal values. The mean corpuscular haemoglobin (MCH) was below the normal range in 35.72 % of the dogs which indicated hypochromic anaemia while in 57.14 % of the dogs its level was above normal indicating macrocytic anaemia. The mean corpuscular haemoglobin concentration (MCHC) was below the normal range in 42.86 % of the dogs indicating anaemia, while in 50 % the MCHC was in normal range. The red blood cells distribution width (RDW %) was below normal in 28.58 % suggesting anisocytosis. This parameter was normal in 21.42 % of the dogs and exceeded the upper limit in 50 % of the animals. The RDWa was below normal limit in 21.43 % of the dogs, within normal range in 57.14 %, and exceeded the upper limit in 21.43 %.

The MPV was increased above normal in 14.28 %, probably indicating subclinical stage of an infection and was in the normal range in 85.72 %. The blood urea nitrogen (BUN) was in the normal range in 78.57 % of the infected dogs and in patient No. 10 (7.14 %) it was below the lower limit. An increased level of the BUN was detected in 14.29 % of the dogs (patients No. 9 and 13), probably because of kidney damage, in conjunction with elevated values of total protein (TP) and creatinine (CRE). The alanine transferase (ALT) was above the normal range in patient No. 12 (7.14 %), in conjunction with decreased TP which is an indication of liver damage [3]. The increased values of ALT indicated liver damage and early detection can be made by this. The total protein values were above normal in patients No. 5 and No. 13 (14.29 %), suggesting subclinical infections. A decreased TP was found in patient No. 12 (7.14 %) which, in conjunction with high ALT, indicated liver damage. Creatinine values were above normal in patient No. 13 (7.14 %) which in conjunction with the BUN and TP values suggested kidney damage. Lower creatinine and BUN was detected also in patient No. 10, indicating kidney failure. Normal values were detected in 85.72 % of the infected dogs. ALP exceeding the upper limit was found in patient No. 14 (7.14 %), suggesting potential liver disease. In patient No. 12, we detected a value of 80 % IU/L which is within the normal range but higher than in the rest of the patients. Normal values were detected in 92.86 % of the dogs. White blood cells and lymphocytes were within the normal range in all dogs. MID and GRAN were above normal in 7.14 % of the infected dogs and normal in 92.86 %.

The haematological results in patients No. 15 — No. 24, negative in the Speed Ehrli test, were within the normal ranges. Platelet values were normal in 100 % of the dogs, while RDW was normal in 70 % and exceeded the upper limit in 30 %. The RBC values were normal in 100 % of dogs, while the haematocrit was normal in 90 % and below the lower limit in 10 % of the normal dogs. All dogs showed normal haemoglobin values. The MCV were normal in 90 % of the dogs and below the normal limit in 10 %. The MCH exceeded the upper limit in 90 % of the dogs and were in the normal range in 10 % of them. The MCHC were normal in all dogs. The MPV were normal in 90 % of dogs and below the lower limit in 10 % of them. The WBC, MID, LYM, GRAN, GLU, ALT, and CRE were in the normal range in 100 % of the dogs. The BUN was within the normal range in 90 % of the dogs, while in 10 % it was below the lower limit. The TP was normal in 60 % and below the normal limit in 40 % of the dogs.

Variations in haematological profiles in *E. canis* infected dogs may be related to: differences in the virulence of the *E. canis* strains, antigen heterogeneity of this bacterial agent, and the clinical form of the disease [4, 5]. Further studies are required to determine the influence of contaminant infections with other bacteria or parasite such as *Leishmania infatum*, *Bartonella* spp., *Babesia* spp., etc., on the observed haematological abnormalities [5, 11, 13, 15]. *Leishmania infatum* has a high prevalence in Lesvos, as well as in the rest of Greece and other Mediterranean countries. According to our study, it seems that the percentage of infection of dogs in Lesvos is high. This can also vary with the season, because the dogs get ticks more often during the summer, from May to October, which was also observed in our study. No official records can be found related to *Ehrlichia canis* infection in Lesvos except for individual private veterinary clinics. Compared with other studies conducted in other parts of Greece or neighbouring countries which have similar weather conditions and harbour ticks

as *R. sanguineus*, our research showed no significant differences [15]. It appears that more preventative measures should be taken by owners of domestic dogs and also by the state for stray dogs which are numerous in Lesvos island.

CONCLUSIONS

Early detection of *E. canis* in dogs is very important, so monitoring and control programs should be established. In Lesvos there was noticed a raised awareness of this disease by local veterinarians and many owners. Serological and preventive measures towards dogs and ticks could help to decrease further the number of infected animals. Our study allowed us to conclude that Speed Ehrli test contributes toward quick diagnosis of symptomatic and asymptomatic patients. From the 24 examined dogs, 14 were found positive. The occurrence of the disease seemed to be more frequent during May-October, which correlates with the timing of ticks being active and also with increased temperatures. The determination of haematological and biochemical parameters detected thrombocytopenia and anaemia in all positive dogs, as well as liver and kidney damage in some of them. A combination of haematological and biochemical values with clinical diagnosis can allow veterinarians to obtain a better clinical picture, administer the best treatment and recommend relevant prophylactic and preventive measures.

REFERENCES

1. **Bowman, D., Susan, E., Lorentzen, L., Shields, J., Sullivan, P., Carlin, E. P., 2009:** Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia cani*s, and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic based serologic survey. *Vet. Parasitology,* 160, 138—148.
2. **Harrus, S., Aroch, I., Lavy, E., Bark, H., 1997:** Clinical manifestations of infectious canine cyclic thrombocytopenia. *Veterinary Record,* 141, 247—250.
3. **Harrus, S., Waner, T., Avidar, Y., Bogin, E., Peh, H., Bark, H., 1996:** Serum protein alterations in canine ehrlichiosis. *Vet. Parasitology,* 66, 241—249.
4. **Harrus, S., Waner, T., 2013**: Canine monocytic Ehrlichiosis – from pathology to clinical manifestation. *Israel Journal of Veterinary Medicine,* 68, 292—296.
5. **Harrus, S., Waner, T., Bark, H., Jongejan, F., Cornelissen, A. W. C. A., 1999:** Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. *J. Clin. Microbiol*., 37, 2745—2749.
6. **Harrus, S., Waner, T., Eldor, A., Zwang, E., Bark, H., 1996:** Platelet dysfunction associated with experimental acute canine ehrlichiosis. *Veterinary Record,* 139, 290—293.
7. **Harrus, S., Waner, T., Weiss, D. J., Keysary, A., Bark, H., 1996:** Kinetics of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. *Vet. Immunol. Immunopathol.,* 51, 13—20.
8. **Kuehn, N., Gaunt, S., 1985:** Clinical and hematological finding in canine ehrlichiosis. *J. Am. Vet. Med. Assoc.,* 186, 355—358.
9. **Lewis, D. C., Meyers, K. M., 1996:** Canine idiopathic thrombocytopenia purpura. *J. Vet. Int. Med.,* 10, 207—218.
10. **Mylonakis, M. E., Koutinas, A. F., Billini,s C., Leontides, L. S., Kontos V., Papadopoulos O., et al., 2003:** Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (*Ehrlichia canis*): A comparison between five methods. *Vet. Microbiology*, 91, 197—204.
11. **Mylonakis, M. E., Koutinas, A. F., Breitschwerdt, E. B., Hegarty, B. C., Billinis, C. D., Leontides, L. S., Kontos, V. I., 2004:** Chronic canine ehrlichiosis (*Ehrlichia canis*): a retrospective study of 19 natural cases. *J. Am. Anim. Hosp. Assoc.,* 40, 174—184.
12. **Mylonakis, M. E., Xenoulis, P. G., Theodorou, K., Siarkou, V. I., Steiner, J. M., Harrus, S., et al., 2014:** Serum canine pancreatic lipase immunoreactivity in experimentally induced and naturally occurring canine monocytic ehrlichiosis (*Ehrlichia canis*). *Vet. Microbiology,* 169, 198—202.
13. **Pusterla, N., Huder, J., Wolfensberger, C., Litschi, B., Parvis, A., Lutz, H., 1997:** Granulocytic ehrlichiosis in two dogs in Switzerland. *J. Clin. Microbiol.*, 35, 2307—2309.
14. **Smith, R. D., Ristic, M., Huxsoll, D. L., 1975:** Platelet kinetics in canine ehrlichiosis: evidence for increased platelet destruction as the cause of thrombocytopenia. *Infection and Immunity,* 11, 1216—1221.
15. **Tsachev, I., Ivanov, A., Dinev, I., Simeonova, G., Kanakov, D., 2008:** Clinical *Ehrlichia canis* and *Hepatozoon canis* coinfection in a dog in Bulgaria. *Revue De Medecine Veterinaire,* 159, 68—73.

Received May 10, 2017
Accepted May 24, 2017