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Short Communication

HAEMATOLOGIC INDICES IN CLINICALLY HEALTHY OUTDOOR DOGS EXPOSED TO VECTOR-BORNE PATHOGENS

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Abstract

Haematologic abnormalities possibly associated with exposure to vector-borne pathogens are rarely reported in clinically healthy outdoor dogs. Therefore, we analysed changes in the complete blood count (CBC) of clinically healthy outdoor dogs seroreactive to *Anaplasma* spp. and *Babesia* spp., with or without microfilariosis. Stray, shelter and hunting dogs, 81 in total, that were polymerase chain reaction negative for *Anaplasma* spp. and *Babesia* spp. were divided into groups according to their seroreactive status and results of a modified Knott's test: seronegative to both *Anaplasma* spp. and *Babesia* spp. SN (*N*=26); seroreactive to *A. phagocythophilum* SR-A (*N*=12); seroreactive to *B. canis*, *B. gibsoni* and/or *B. vogeli* SR-B (*N*=25); and seroreactive to both of the pathogens SR-AB (*N*=8). These four groups were negative to microfilariosis, unlike the fifth group, seroreactive to either or to both of the pathogens and with microfilariosis SR-M (*N*=10). The frequencies of CBC alterations among all analysed dogs were: 0.35 – leucocytosis, 0.44 – granulocytosis, 0.28 – anaemia, 0.74 – microcytosis, 0.37 – increased mean cell haemoglobin concentration (MCHC) and 0.33 – thrombocytopenia. The frequency of alterations did not differ across the groups. An exception was the SR-M group wherein increased MCHC peaked with a frequency of 0.80, while in the

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other four groups, the frequency ranged between 0.10 and 0.50. Clinically healthy outdoor dogs have multiple CBC abnormalities, consistent with stress and low-level chronic inflammation, but not associated with a previous exposure to *Anaplasma* spp. or *Babesia* spp. The presence of microfilaria increases haemolysis *in vitro*.

Key words: Anaemia, *Anaplasma* spp., *Babesia* spp., leucocytosis, thrombocytopenia, dogs

INTRODUCTION

The epidemiology of canine vector-borne pathogens (VBPs) is commonly investigated in asymptomatic outdoor dogs in Serbia (Gabrielli et al., 2015; Obrenović et al., 2015). Often, the studies included shelter, but also stray and hunting dogs (Spasojević-Kosić et al., 2015). Exposure to various vectors and VBPs is rather common in these dogs because they spend most of the time in a natural environment without any appropriate protection against vectors and parasites. The study of Kovačević Filipović et al. (2018) conducted in Serbia showed that a population of outdoor dogs were a significant source of potential hosts for several VBPs.

Although studies on prevalences of VBPs in clinically healthy dogs are numerous, only some of them assessed the health status of the animals (Spasojević-Kosić et al., 2015). Apart from the possible specific pathogenic effect that is associated with blood cell tropism of VBPs, it is highly possible that frequent exposure to vectors and pathogens causes low-grade tissue damage that could lead to an asymptomatic chronic inflammation (Otranto et al., 2009). Thus, it could be assumed that, as a consequence, haematologic changes consistent with a chronic inflammation, *e.g.* mild leucocytosis, microcytic anaemia and variable findings in the number of thrombocytes, could be induced.

Our aim was to investigate the frequency of the above-mentioned changes in the complete blood count (CBC) of clinically healthy outdoor dogs that were polymerase chain reaction (PCR) negative but seroreactive to *Anaplasma* spp., *Babesia* spp., with and without microfilariosis.

MATERIALS AND METHODS

We used the surplus of venous blood samples collected from clinically healthy stray, hunting and shelter dogs belonging to a larger group formed for our previous study on molecular detection and seroprevalence of *Anaplasma phagocytophilum*, *A. platys*, *Ehrlichia canis*, *E. chaffeenses*, *E. ewingii*, *Borrelia burgdorferi*, *Babesia canis*, *B. gibsoni*, and *B. vogeli* (Kovačević Filipović et al., 2018). Tick/Vector Comprehensive Real PCR Panel Canine (IDEXX Laboratories, Westbrook, Maine) was used for the molecular detection of the previously named pathogens. Seroreactivity was tested with an immunofluorescence antibody test (Megafluo®Vet, Megacor Diagnostik GmbH, Austria) or SNAP® 4Dx Plus technology (Kovačević Filipović et al., 2018). Eligible animals were PCR negative

to the investigated pathogens and seronegative to *Borrelia* spp. and *Ehrlichia* spp. The selected dogs (*N*=81) were divided into five groups according to their serological status and results of a modified Knott's test (MKT):

1. SN (*N*=26): seronegative to *Anaplasma* spp. and *Babesia* spp. and negative MKT,
2. SR-A (*N*=12): seroreactive only to *A. phagocythophilum* and negative MKT,
3. SR-B (*N*=25): seroreactive to *B. canis*, *B. gibsoni* and/or *B. vogeli*, and negative MKT,
4. SR-AB (*N*=8): double or triple seroreactive to *A. phagocythophilum* and to at least one of the *Babesia* species: *B. canis*, *B. gibsoni* and/or *B. vogeli*, and negative MKT, and
5. SR-M (*N*=10): miscellaneous seroreactivity with positive MKT. Among these dogs one was seroreactive only to *A. phagocythophilum*, six to *B. canis* and three were double seroreactive to *A. phagocytophilum* and *B. canis*.

The venous blood samples in tubes with EDTA, collected for PCR analysis, were also used to determine the CBC (Abacus Junior Vet, Diatron, Vienna, Austria) and to perform MKT as described by Magnis et al. (2013) within two hours of collection.

Statistical analysis included Chi-square test with the Boniferroni correction for multiple comparisons (MedCalc® software version 16.2.1), where P-value less than 0.05 was considered significant.

In accordance with the European Union declaration 63/2010 and based on the Serbian Animal Welfare Law, we obtained a permission for this study from the Ministry of Agriculture, Forestry and Water Management (number 323-07-03455/2015-05/3) prior to its commencement. Furthermore, we collected the blood samples from shelter and hunting dogs with the informed consent of responsible shelter management personnel or the dog owners.

RESULTS AND DISCUSSION

Our study focuses on the changes in CBC in dogs with different serological status against *Anaplasma* spp. and *Babesia* spp., with or without the presence of microfilaria.

First, we analysed total leukocyte number. Over all five dog groups, leucocytosis (Figure 1A) and increased granulocyte counts (Figure 1B) had a frequency of 0.35 and 0.44, respectively. Lymphopenia was present with frequency of 0.22, while lymphocytosis (frequency 0.05) was a rather rare finding (Figure 1C). The alterations in the number of monocytes and part of eosinophils (denoted jointly as medium (MID) cells) were minimal, i.e., the frequency of decreased count was 0.10, while an increased count was encountered in only one dog (Figure 1D). This finding is in accordance with a previous study in hunting dogs seropositive to VBPs (Spasojević-Kosić et al., 2015). Our result showing an increased granulocyte count in combination with lymphopenia probably reflects exposure to different environmental stressors in addition to the evident exposure to ticks and tick-borne pathogens. We suppose that some of the environmental stressors for outdoor dogs could be quantitative and/or

qualitative nutrition deficiencies, exposure to bad weather conditions, parasite load and even a lack of appropriate social relationships (Uetake et al., 2016). However, it is clear thatthe presence of *Anaplasma* spp., *Babesia* spp. and microfilaria is not a major stressor in the population of outdoor dogs, as the change in neutrophil and lymphocyte number is unequivocally present in all our investigated groups.

When all five groups were analysed together, the decreased number of red blood cells (RBC) was also a rare finding, as illustrated with frequency of 0.07 (Figure 2A).

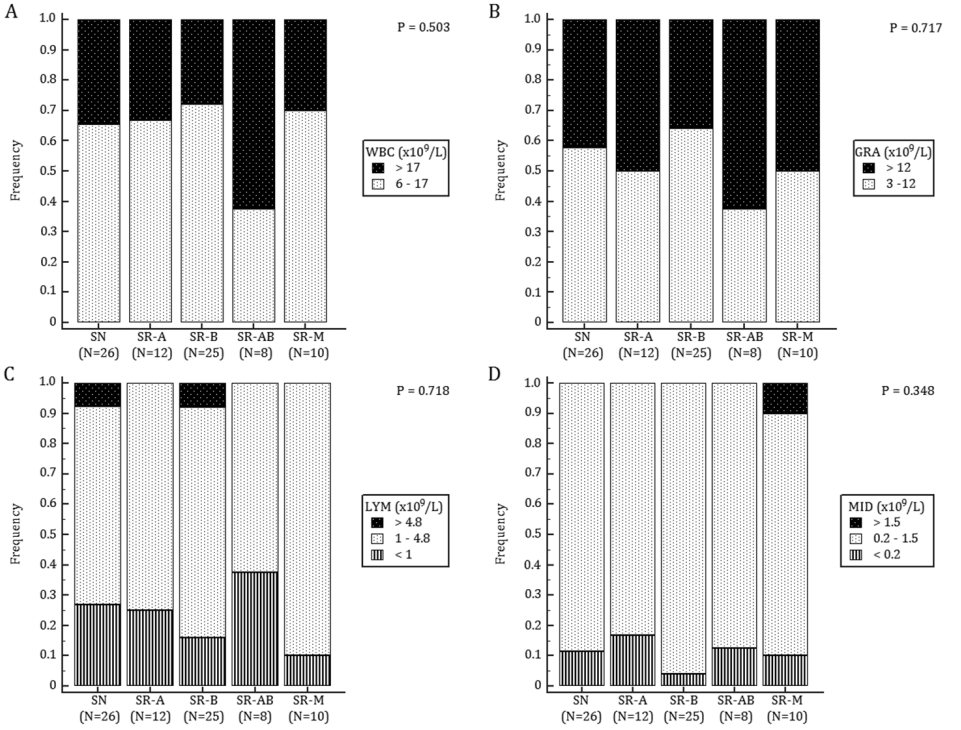

Figure 1. Frequency of changes in total (**A**) and differential leukocyte count **(B-D)**: WBC – total leukocyte count; GRA – granulocyte count; LYM – lymphocyte count; MID – medium cell (monocytes and part of eosinophils) count. Reference intervals for each count are given in the light dotted bars, lower values in grey and higher values in black bars. SN – seronegative; SR-A – seroreactive to *Anaplasma* spp.; SR-B – seroreactive to *Babesia* spp.; SR-AB – seroreactive to *Anaplasma* spp. and *Babesia* spp.; SR-M – seroreactive to either or to both *Anaplasma* spp. and *Babesia* spp. and with microfilariosis.

A similar situation was noticed for haemoglobin (HGB), as concentrations below the reference range occurred with a frequency of 0.05 (Figure2B). Anaemia among all dogs occurred at a frequency of 0.28, using the haematocrit (HTC) value below 0.37 as the cut-off (Figure 2C). This finding together with the microcytosis frequency of 0.74 (Figure 3A) may indicate anaemia due to chronic inflammation was present in the

population of investigated dogs. The other possibility is a nutritional deficiency, which at present cannot be proved.

Among all investigated dogs, increased mean cell haemoglobin concentration (MCHC) was present with the frequency of 0.37. Nevertheless, it was the only parameter that showed a significant difference between the groups (Figure 3B). The prevalence of dogs that had an increased MCHC was higher in the SR-M group (0.80) than in the SR-A (0.10), SR-B group (0.24), SR-AB group (0.24) and SN group (0.50). This finding is consistent with the study of Milanović et al. (2017), which demonstrated that in dogs with acute phase reaction due to *B. canis* infection, haemolysis was more pronounced when the dogs were concurrently infected with *Dirofilaria immitis*. Furthermore, there is also a possibility that blood sampling potentiates some degree of haemolysis in erythrocytes that already have an increased osmotic fragility due to microfilariosis.

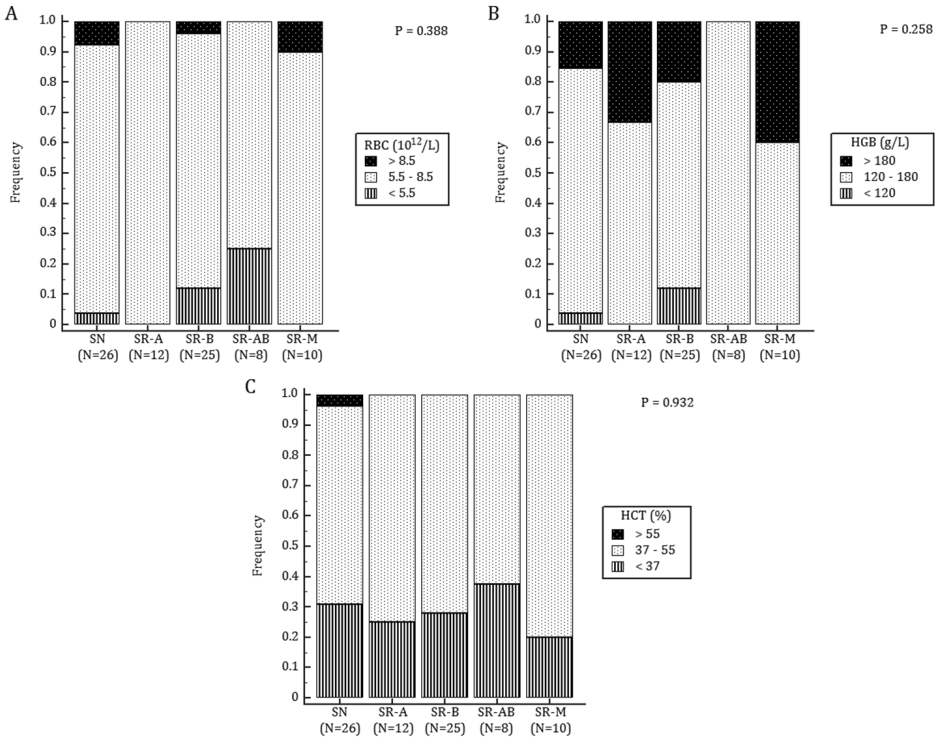

Figure 2. Frequency of changes in number of erythocytes **(A)**, concentration of haemoglobin **(B)** and haematocrit **(C)**. Reference intervals for each parameter are given in the light dotted bars, lower values in gray and higher values in black bars (RBC –number of erythocytes; HGB – haemoglobin concentration; HCT – haematocrit). SN – seronegative; SR-A – seroreactive to *Anaplasma* spp.; SR-B – seroreactive to *Babesia* spp.; SR-AB – seroreactive to *Anaplasma* spp. and *Babesia* spp.; SR-M – seroreactive to either or to both *Anaplasma* spp. and *Babesia* spp. and with microfilariosis.

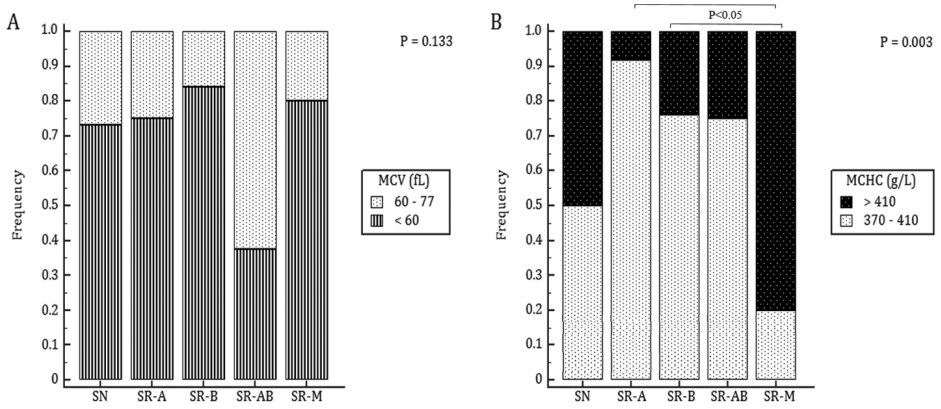

Figure 3. Frequency of changes in mean corpuscular volume **(A)** and mean cell haemoglobin concentration **(B)**. Reference intervals for each parameter are given in the light dotted bars, lower values in grey and higher values in black bars (MCV – mean corpuscular volume; MCHC – mean cell haemoglobin concentration). SN – seronegative; SR-A – seroreactive to *Anaplasma* spp.; SR-B – seroreactive to *Babesia* spp.; SR-AB – seroreactive to *Anaplasma* spp. and *Babesia* spp.; SR-M – seroreactive to either or to both *Anaplasma* spp. and *Babesia* spp. and with microfilariosis.

Finally, thrombocytopenia was encountered in one third (0.33) of all the dogs (Figure 4), pointing to the fact that thrombocytopenia is not more frequent among dogs exposed to *Anaplasma* spp. and *Babesia* spp. than among non-exposed dogs. Since thrombocytopenia is the most frequent haematologic abnormality in veterinary medicine (Zimmerman, 2000), this finding was not unexpected, but its true nature remains unknown.

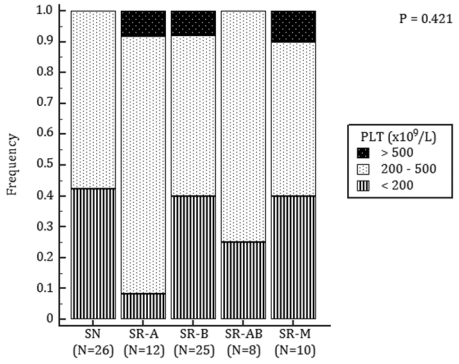

Figure 4. Frequency of changes in platelet count. Reference interval is given in the light dotted bars, lower values in grey and higher values in black bars (PLT – platelet count). SN – seronegative; SR-A – seroreactive to *Anaplasma* spp.; SR-B – seroreactive to *Babesia* spp.; SR-AB – seroreactive to *Anaplasma* spp. and *Babesia* spp.; SR-M – seroreactive to either or to both *Anaplasma* spp. and *Babesia* spp. and with microfilariosis.

Still, we cannot rule out the possibility the dogs were infected with agents not investigated in our study. For example, golden jackals in the wilderness in Serbia, apart from being reservoirs of *Anaplasma* spp. and *Babesia* spp. (Sukara et al., 2018), can also be infected with *Brucella* spp. and *Leishmania* spp. (Ćirović et al., 2014), and probably a number of other pathogens that could trigger subclinical inflammatory response. Furthermore, in general, ticks in Serbia are infected with *Rickettsia* spp., *Canididatus* Neoehrlichia mikurensis and *Hepatozoon canis* (Potkonjak et al., 2016), all of which could be transmitted to dogs, and induce, at least, mild CBC abnormalities, similar to those found in our study. More complex analysis of the dogs' microenvironment could identify the exact stressors. Additionally, thorough investigation of the ectoparasites and endoparasites present in the dogs as well as possible co-infections and co-morbidities could give amore precise information about the dogs' overall health and the reasons for the observed CBC changes.

CONCLUSION

The population of clinically healthy outdoor dogs shows multiple CBC abnormalities that are consistent with stress and low-level chronic inflammation, but are not associated with previous exposure to *Anaplasma* spp., *Babesia* spp., or the presence of microfilaria.

Acknowledgements

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Authors' contributions

FJ, ZM, AIB, NA and KS performed data collection and designed the study. FJ, AB and MKF carried out the statistical analysis. FJ, AB, JA and MKF wrote the manuscript.

Competing interests

The authors: Filip Janjić, Zorana Milanović, Anja Ilić Božović, Nenad Andrić, Kristina Spariosu, Jelena Ajtić, Andjelo Beletić, Milica Kovačević Filipović declare that they have no competing interests.

REFERENCES

Ćirović D., Chochlakis D., Tomanović S., Sukara R., Penezić A., Tselentis Y., Psaroulaki A. 2014. Presence of *Leishmania* and *Brucella* species in the golden jackal *Canis aureus* in Serbia. BioMed Research International, 28516. http://dx.doi.org/ 10.1155/2014/728516.

Filipović-Kovačević M., Beletić A., Božović-Ilić A., Milanović Z., Tyrrell P., Buch J., Breitschwerdt E. B., Birkenheuer A. J., Chandrashekaret R. 2018. Molecular and serological prevalence of Anaplasma phagocytophilum, A. platys, Ehrlichia canis, E. chaffeenses, E. ewingii, Borrelia burgdorferi, Babesia canis, B. gibsoni and B. vogeli among clinically healthy

outdoor dogs in Serbia. Veterinary Parasitology, Regional Studies and Reports, 14:117-122. http://dx.doi.org/10.1016/j.vprsr.2018.10.001.

Gabrielli S., Otašević S., Ignjatović A., Savić S., Fraulo M., Arsić-Arsenijević V., Momčilović S., Cancrini G. 2015. Canine babesioses in noninvestigated areas of Serbia. Vector-Borne and Zoonotic Diseases, 15:535-538. http://dx.doi.org/10.1089/vbz.2015.1797.

Magnis J., Lorentz S., Guardone L., Grimm F., Magi M., Naucke T. J., Deplazes P. 2013. Morphometric analyses of canine blood microfilariae isolated by the Knott's test enables Dirofilaria immitis and D. repens species-specific and Acanthocheilonema (syn. Dipetalonema) genus-specific diagnosis. Parasites & Vectors, 6:48. http://dx.doi.org/10.1186/1756-3305-6-48.

Obrenović S., Ristanović E., Čekanac R, Radulović Ž., Ilić V. 2015. Seroprevalence of IgG antibodies against Borrelia burgdorferi in dogs in Belgrade area, Serbia. Acta veterinaria-Beograd, 65(1):99-110.

http://dx.doi.org/10.1515/acve-2015-0008.

Otranto D., Dantas-Torres F., Breitschwerdt E. B. 2009. Managing canine vector-borne diseases of zoonotic concern: part two. Trends Parasitol, 25:228-235.

http://dx.doi.org/10.1016/j.pt.2009.02.005

Potkonjak A., Gutiérrez R., Savić S., Vračar V., Nachum-Biala Y., Jurišić, A., et al. 2016. Molecular detection of emerging tick-borne pathogens in Vojvodina, Serbia. Ticks Tick Borne Dis, 7:199-203.

http://dx.doi.org/10.1016/j.ttbdis.2015.10.007.

Spasojević-Kosić L., Savić S., Potkonjak A., Vračar V. 2015. Retrospective analysis of clinical and laboratory findings in hunting dogs with serologic reactions to tick-borne pathogens (Anaplasma phagocytophilum, Borrelia burgdorferi, Babesia canis, Ehrlichia canis, Rickettsia conorii). Veterinarski Glasnik, 69:219-232.

http://dx.doi.org/10.2298/VETGL1504219

Sukara R., Chochlakis D., Ćirović D., Penezić A., Mihaljica D., Ćakić, S., Vlačić M., Tselentis Y., Psaroulaki A., Tomanović S. 2018. Golden jackals (Canis aureus) as hosts for ticks and tick-borne pathogens in Serbia. Ticks and Tick-borne Diseases, 9(5):1090-1097. http://dx.doi.org/j.ttbdis.2018.04.003.

Uetake, K., Uchida, H., Ishihara, J., Kushima, S., Tanaka, T. 2015. Faecal corticosterone levels of dogs relinquished to a shelter in Japan. Veterinary Record open, 2(2), e000139. http://dx.doi.org/10.1136/vetreco-2015-000139

Zimmerman K. L. 2000. Chapter 67: Drug-Induced Thrombocytopenias. In Schalm's Veterinary Haematology (Fifth Edition). Eds. B. F. Feldman, J. G. Zinkl, N. C. Jain, Lippincott Williams & Wilkins, Philadelphia, pp. 472.

HEMATOLOŠKI PARAMETRI KOD KLINIČKI ZDRAVIH PASA KOJI BORAVE NA OTVORENOM I IZLOŽENI SU PATOGENIMA KOJE PRENOSE VEKTORI

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Kratak sadržaj

Klinički zdravi psi koji borave na otvorenom i koji su izloženi patogenima koje prenose vektori mogu imati hematološke premećaje vezane za dejstvo tih patogena. Ipak, hematološke pretrage se retko izvode na toj populaciji pasa. Stoga je cilj našeg rada bio da utvrdimo promene u krvnoj slici zdravih pasa lutalica, pasa smeštenih u azilima i lovačkih pasa, seroreaktivnih na uzročnike bolesti iz rodova *Anaplasma* i *Babesia*, sa ili bez mikrofilarioze. U ovom radu je ispitana krvna slika 81 psa. U studiju su uključeni psi kod kojih lančanom reakcijom polimerizacije nije pokazano prisustvo DNK *Anaplasma* spp. i *Babesia* spp. Psi su prema serološkom statusu raspoređeni u sledeće grupe: seronegativni na *Anaplasma* spp. i *Babesia* spp. SN (*N*=26), seroreaktivni na *A. phagocytophilum* SR-A (*N*=12), seroreaktivni na *B. canis*, *B. gibsoni* i/ili *B. vogeli* SR-B (*N*=25) i seroreaktivni na oba navedena patogena SR-AB (*N*=8). Ove četiri grupe su bile negativne na mikrofilariozu, dok je peta grupa, seroreaktivna na neki ili oba patogena imala i mikrofilarije SR-M (*N*=10). Učestalost promena kompletne krvne slike među svim ispitivanim psima je bila sledeća: 0,35 za leukocitozu, 0,44 za granulocitozu, 0,28 za anemiju, 0,74 za mikrocitozu, 0,37 za povećanu prosečnu koncentraciju hemoglobina u eritrocitima i 0,33 za trombocitopeniju. Grupe se nisu razlikovale u pogledu učestalosti navedenih promena. Izuzetak je grupa SR-M u kojoj je učestalost povećanja vrednosti za prosečnu koncentraciju hemoglobina u eritrocitima iznosila 0,80. U ostale četiri grupe ta učestalost je iznosila između 0,10 i 0,50. Zaključak je da ispitivani psi imaju višestruke poremećaje krvne slike koji najverovatnije odražavaju stres i nizak stepen hronične inflamacije, ali ne i vezu sa prethodnim kontaktom sa *Anaplasma* spp. i *Babesia* spp. Prisustvo mikrofilarija izaziva hemolizu *in vitro*.

Ključne reči: anemija, *Anaplasma* spp., *Babesia* spp., leukocitoza, trombocitopenija, psi