Blood Morphology in Dogs with Dirofilariasis

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Abstract. The article addresses the results of a study of blood morphology parameters in healthy dogs vs. dogs diagnosed with dirofilariasis. In the group of infected animals, the most significant difference was 4.9 higher granulocytes. Another significant contribution is eosinophils and, to a lesser extent, neutrophils and monocytes. In blood of infected and healthy animals, no difference was found in terms of the count of red blood cells, haemoglobin, and haematocrit. Anisocytosis value in red blood cells is probably related to the compensated processes, and differs insignificantly between healthy and infected animal groups. Blood morphology of infected animals is characterized by mild reticulocytosis, not exceeding 20% of physiological norm. In the group of infected dogs, platelet count corresponds to the lower boundary of physiological norm. In healthy animals' group, platelets and platelet crit is physiologically normal. Leukopenia, lymphocytopenia are detected. Apparently, the reason behind the findings is age of the animals and degree of their adaptation to the progress of the invasive process.

1 Introduction

Canine vector-borne diseases (CVBD) are gaining an increasing importance. Causative agents and transmitters of these diseases (fleas, mosquitoes, ticks and sandflies) are ubiquitous. Notwithstanding the efforts of the practical veterinary medicine, the spread of ectoparasites is universal.

In the Russian Federation, dirofilariasis is studied by a number of science centers and research teams. The spread of dirofilariasis has long been treated as a problem of the North-West region of Caucasus [1]. Recently the veterinary services are registering a growth in incidence of invasion in dogs inhabiting more temperate latitudes.

Since 2000, the research has reflected a growth in the number of dogs invaded by the two dirofilariasis causative agents: *Dirofilaria immitis* and *Dirofilaria repens*. According to different sources, the spread of dirofilariasis is within 9 to 13-16.6 %

The problem transcends the boundaries of veterinary medicine. Since 2006, Rospotrebnadzor (Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing) registers the growth of dirofilariasis incidence among the population of the Russian Federation. There are several causes to this: dirofilariasis is

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transmitted via bites of several mosquitoes: *Aedes (Stegomyia)*, *Culex*, *Anopheles*, *Coquillettidia (Mansonia)* [3], out of which 70 types of mosquitoes are capable of maintaining the development of larvae of *Dirofilaria immitis*, and 20 types of mosquitoes can act as bridging hosts of *Dirofilaria repens*. In some instances, mixtinvasions are registered [4-8]. Parasite fauna of the region is formed under the influence of many climatic, geographic, and socio-economic factors. To a large extent, dirofilariasis spread is facilitated by anthropogenic contributors, e.g. presence of stagnant water bodies, as well as territories with high dog populations [9].

The research conducted in Russia over the recent decades for study of parasite fauna in the population of wild vertebrates of the families of Canidae and Felidae shows high rates of invasion in carnivores with both *Dirofilaria immitis* (Leidy, 1856) and *Dirofilaria repens* (Railliet et Henry, 1911) [10;11,12,13, 14].

Dirofilaria infection rate in wild animals inhabiting the South of Russia remains high. According to the literature, in Krasnodar Krai, out of the 60 tested jackals, *Dirofilaria immitis* helminths were found in 14 (23,3%), *Dirofilaria repens* helminths in 6 (10%), and mixtination with *D. immitis* and *D. repens* in 4 (6.7%) animals. Invermination with this species is also found in raccoon dogs, badgers, foxes and leopard cats [11,12,13, 15].

Incidence of *D. repens* and *D. immitis* infection in foxes has been examined in various eco-geographic zones of the Central Non-Black Earth Belt of Russia: in Moscow, Ivanov, Vladimir, Kostroma, Smolensk, Ryazan and Yaroslavl Oblasts [10, 14]. The obtained data shows that in the conditions of anthropogenic systems (agricultural landscapes, territories adjacent to human settlements) wild animals participate in circulation of zoonotic helminthiasis. Wild animals play the role of the main ecological link – as the source, accumulation and spread factor of dirofilariasis causative agents [16]

Dirofilariasis is one of the most dangerous invasive diseases in dogs. Invasion caused by *D. Immitis* is the most critical one.

A specific relation has been observed between service use of dogs and the risk of dirofilariasis infection. Further analysis of dirofilariasis incidence dynamics in dogs and their use over years has shown that the invasion tends to be the most extensive in homeless (unsupervised) and service dogs. No examination or treatment is performed in regard to unsupervised animals. Service dogs, on the other hand, undergo annual scheduled diagnostic testing, prevention and treatment of dirofilariasis [17]. German Shepherds, East-European Shepherds, Caucasian Shepherds, Central Asian Shepherds, Doberman Pinchers, Giant Schnauzers and Boxers are breeds that are most susceptible to dirofilariasis infection [18, 19]. The most vulnerable dog ages are 4 to 6 years old; according to some sources, high risk of infection remains until 8 years of age [9].

At the same time, in hunting and domestic animals, this invasion is usually diagnosed incidentally. Preventive treatments are performed by animal owners sporadically, since para. 9.4. of Sanitary and epidemiological regulations and norms 3.2.3215-14 specifies the need to examine and anthelmintic treatment domestic animals without listing specific drugs and frequency of their use [17].

Due to the prevailing population of German Shepherds in the security forces of the Russian Federation, the problem of dirofilariasis spread is highly relevant. Research of the invasion process dynamics has shown seasonality of the disease which grown considerably within the mosquito breed period in summer.

2 Materials and Methods

In case of dog infection with *Dirofilaria immitis*, 3 forms of the disease in terms of clinical symptoms manifestation are distinguished. The clinical stage of dirofilariasis development correlates with the number of mature helminths. The first stage is a subclinical form of the

disease and has next to no apparent clinical symptoms. Medium severity degree of the disease manifests as a cardiopulmonary process with renal insufficiency. The most severe form is accompanied by systemic changes in the body (cardiopulmonary disease with thromboembolic complications, accompanied by renal and hepatic insufficiency) [18]. Invasion process caused by *Dirofilaria repens* can manifest in 3 forms: subclinical, dermal and the most severe pseudo-tumorous form of the disease [19].

We have performed diagnosis of dirofilariasis and hematological analysis in healthy and infected dogs [20].

Notably, infection with dirofilariasis is accompanied with significant decrease in physical stamina of dogs, their olfactory activity, and ability to perform service and combat tasks. Most dogs eventually die of thromboembolism and cardiovascular complications. The research involved German Shepherd and East European Shepherd breeds with a total headcount of 98 animals. The results of blood morphology study are shown in the Table below.

Table 1.Blood morphology parameters in dogs

Parameters	Infected animals	Healthy animals
WBC (leucocytes)	9.10 ± 1.41	11.20 ± 1.20
LYM (lymphocytes)	3.20 ± 0.54	7.63 ± 0.61
MON (monocytes)	1.05 ± 0.09	0.80 ± 0.14
GRA (granulocytes)	5.40 ± 0.96	1.70 ± 1.06
LYM%(lymphocytes %)	32.6 ± 4.06	73.90 ± 5.77
MON%(monocytes %)	10.75 ± 0.74	7.80 ± 0.62
GRA%(granulocytes %)	56.00 ± 4.57	17.30 ± 5.31
RBC (red blood cells)	6.95 ± 0.34	7.07 ± 0.21
HGB (haemoglobin)	16.45 ± 0.57	17.40 ± 0.60
HCT (haematocrit)	42.00 ± 1.51	44.10 ± 1.48
MCV (Mean Cell Volume)	59.85 ± 0.98	62.90 ± 0.67
MCH (Mean Cell Haemoglobin)	23.80 ± 0.45	23.40 ± 0.67
MCHC (Mean Corpuscular Haemoglobin Concentration)	39.45 ± 0.31	37.00 ± 0.30
RDWCV (Red blood cell distribution width)	14.95 ± 0.31	14.80 ± 0.37
RDWC% (Red blood cell distribution width, %)	28.20 ± 1.22	34.30 ± 0.80
PLT (Platelets)	141.50 ± 21.60	160.00 ± 13.24
MPV (Mean Platelets Volume)	9.05 ± 0.31	8.60 ± 0.12
PCT (Platelets Cell)	0.124 ± 0.017	0.13 ± 0.01
PDV (Platelets width)	18.20 ± 0.97	17.70 ± 0.72
PLCR (Platelets Large Cell ratio)	20.05 ± 2.77	18.10 ± 0.98

The blood morphology parameters were examined using IDEXX Laser Cyte automatic analyzer.

Against the invasion background, changes in the blood morphology of dogs are accompanied by a decrease in red blood cells, minor decrease in concentration of haemoglobin, a decrease in the number of leucocytes and significant eosinophilia. Biochemical parameters of blood are characterized by hyperproteinemia, increasing activity of AST, ALT enzymes, an increase in the levels of bilirubin and urea [22, 23].

Dirofilariasis diagnosis is based on the analysis of epizootological situation, clinical manifestations, as well as the results of laboratory tests for presence of microfilaria in dog blood.

Several methods exist for diagnosis of dirofilariasis. The most common ones are the method of thick blood smear with further microscopic examination, Knott’s method [24], Yastreb’s method (concentration with distilled water) [25], and filtration method [26]. More

reliable methods are ELISA test (enzyme-linked immunosorbent assay) and specific antigen-based immunodiagnosis reaction [27].

It is of special interest to look into using test systems for detection of *Dirofilaria immitis* in dogs, including its amicrofilaremic form, where diagnosis is presently extremely complicated. Use of test systems produced in the US and Korea, usually as an express method, significantly facilitates detection of invasion, however, only in regard to one causative agent type – *D. immitis*. In regard to the other type, *D. repens*, laboratory methods of clinical diagnosis boil down to serological tests and PCR. The capabilities of instrumental diagnosis methods, such as echocardiography, chest radiology, electrocardiography, ultrasonic test (UT) ensure reliable determination of invasion extension in dogs [28].

3 Results and discussion

The analysis of blood morphological parameters such as haematocrit, haemoglobin, red blood cell count, shows that there is less than 10% difference in these parameters between the groups of healthy and infected animals. We assume that compensated hemolysis causes a decrease in red blood cell count, level of haemoglobin, and red blood cells mean volume. Anisocytosis of red blood cells (RDWC) in infected and healthy animals differs between the groups and insignificantly from physiological norm, which amounts to $11.9\text{--}16 \times 10^3/\text{L}$.

Significant differences between the groups of infected and healthy dogs are observed in terms of the level of granulocytes (in absolute values and in percent of the total ratio between granulocytes and agranulocytes). A significant contribution in the increase of granulocyte count is probably made by eosinophils, as well as neutrophils, and to lesser extent monocytes.

Blood morphology manifests with minor reticulocytosis, not exceeding 20% of physiological norm (MCHC), amounting to 32–38 g/L.

In terms of the platelet count, parameters of infected dogs are approaching the lower limit of physiological norm (PLT – $190\text{--}530 \times 10^9/\text{L}$). In the group of healthy dogs, platelet count and platelet crit are physiologically normal.

It was found that monocytes (in % and in total count) in the healthy group correspond to the physiological norm (MON 3–10 %), whereas in the infected group the result is slightly higher than the norm.

In order to control dirofilariasis incidence in service dogs, prevention measures include acaricide drugs.

Use of moxidectin-based drugs enables effective prevention. Literature describes a reaction of hepatic marker enzymes increase: alanine aminotransferase (ALT), aspartate aminotransferase (AST) [28]. Use of anthelmintic drugs is recommended to be accompanied with hepatoprotective medications preventing fatty liver disease. The cytoprotective activity mechanism lies in the unique ability of antioxidants to prevent free-radical oxidation of hepatocyte membranes. The role of hepatoprotectors is important as a means of preventive therapy in order to decrease toxic effects of antigens produced by dirofilaria [29].

The veterinary activity regulations according to Sanitary and epidemiological regulations and norms 3.2.3215-14, Section IX, stipulate the procedure for prevention of dirofilariasis. The action plan consists of extermination of mosquitoes, identification and anthelmintic treatment of invaded dogs, prevention of contact between mosquitoes, humans and animals. All actions include both veterinary and general aspects, e.g. arrangement of dog walking placed away from water bodies where mosquitoes usually breed. In dirofilariasis foci, where high invasion intensity is found in dogs, prevention

steps include thorough treatment of water bodies (delarvation) and treatment of residential and non-residential premises with insecticides [30].

Other promising methods include extermination of mosquitoes using long-action repellents: imagocides, larvicides, as well as barrier methods for protection of humans and animals. One of the most prospective and relevant ones is permethrin, a pyrethroid manifesting high effectiveness and providing protection from many insects [31].

Dirofilariasis prevention in service dogs consists of a set of preventive measures to avoid infection: treatment of animal fur with insect- and acaricide substances, use of anthelmintic treatment and hepatoprotective drugs. Import substitution and cost saving is achieved through use of native counterparts based on fipronil and moxidectin. The cost of preventive drugs is times less than the cost of treatment of infected animals.

4 Conclusion

To conclude, invasion, and specifically development of dirofilariasis, causes insignificant changes in the blood morphology. It was discovered that dirofilariasis invasion is not always accompanied by high RBC count. Leukopenia, lymphocytopenia were found. The blood had high counts of granulocytes (due to eosinophils) influencing the antigen structure of dirofilaria. Apparently, the cause of changes was the age of animals and degree of the invasive process.

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