Hematological parameters of dogs for parasitism *Dirofilaria immitis*

D. Kryvoruchenko

State Biotechnological University, Alchevsky Str., 44, Kharkiv, 61002, Ukraine

Abstract

The relationship between helminths and the host is built on a subtle molecular biological basis. Their pathogenic effect is characterized by the mechanical, toxic, inculating effect on the host organism and the state of reactivity of his organism, immunological and allergic reorganization. It has been proved that helminths of the species *Dirofilaria immitis* are the most pathogenic nematodes of this genus because the localization of the imaginal stage of the parasite causes more complex pathogenesis of the invasion. The study aimed to establish changes in hematological parameters of dogs infected with *D. immitis*, considering the intensity of microfilarias invasion. Studies have shown that the intensity of the invasion of up to 20 larvae/cm² in the blood of dogs, significant changes in the number of erythrocytes, leukocytes, platelets, hemoglobin, and hematocrit and ESR were not found. The intensity of the invasion of 20–40 larvae/cm² in infected dogs in the blood decreases the number of erythrocytes by 11.92 % (P < 0.05), the hemoglobin content by 16.33 % (P < 0.05), the hematocrit by 12.70 % (P < 0.05) and the number of leukocytes increases by 15.17 % (P < 0.05). Indicators of the intensity of invasion of 40–60 larvae/cm² in infected dogs showed more significant changes in the blood, which are characterized by a decrease in the number of erythrocytes by 25.54 % (P < 0.001), platelets by 34.03 % (P < 0.01), hemoglobin content by 30.86 % (P < 0.001), hematocrit by 20.56 % (P < 0.001) and an increase in the number of leukocytes by 32.75 % (P < 0.001) and ESR by 1.84 times (0.001). At indicators of the intensity of invasion of more than 60 larvae/cm², in dogs significant (P < 0.001) decrease in the number of erythrocytes by 47.21 %, thrombocytes by 42.84 %, the content of hemoglobin by 42.27 %, the indicator of hematocrit by 45.57 % and an increase in the number of leukocytes by 44.98 % and ESR by 2.51 times. In the leukoform of dogs with increasing rates of microfilariae invasion, the number of eosinophils gradually increases to 62.38 % (P < 0.001), rod-shaped neutrophils to 27.48 % (P < 0.05), monocytes to 23.21 % (P < 0.01) and the number of lymphocytes is reduced to 45.45 % (P < 0.001). The data obtained indicate the importance of determining these indicators for heartworm disease, which complements the picture of clinical examination of animals and will allow more effective treatment of dogs in this invasion.

Keywords: dogs, dirofilariasis, *Dirofilaria immitis*, hematological indicators intensity of invasion, microfilariae.

1. Introduction

It is known that heartworm disease is common on most continents of the globe. However, the main distribution areas of the invasion are countries with warm and temperate climates and tropical areas around the world, including southern Europe (Dantas-Torres & Otranto, 2013; Genchi et al., 2019; Genchi & Kramer, 2020; Younes et al., 2021).

According to the literature, several species of heartworms have been registered and described in dogs and other carnivores, among which the most common and pathogenic species is *Dirofilaria immitis* Leidy, 1856. This is due to the localization of these parasites in the right ventricle and pulmonary arteries. Severe disorders of all body systems, especially cardiovascular, can cause animal death (Hoch & Strickland, 2008; Maerz, 2020; Romano et al., 2021).

It has been proved that the criterion of pathogenic action of parasites on animals is significant changes in the blood, the results of which can provide data on the state of the body and the course of the disease (Ali et al., 1985; Still & Konråd, 1985; Jiménez-Penago et al., 2021).

According to studies by many researchers, dogs infected with *D. immitis* have found changes in their hematological parameters, which are characterized by the development of anemia, leukocytosis, and accelerated erythrocyte sedimentation rate (Boreham & Atwell, 1983; Goggin et al., 1997; Sevimli et al., 2007).

The authors found that the main hematological changes in dogs with microphilaremia were anemia from mild to moderate, from mild to severe thrombocytopenia, severe leukocytosis, neutrophilia, eosinophilia, and monocytosis. Changes in the serum biochemical parameters of infected dogs were characterized by increased activity of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase (Nivetpathonwat et al., 2007). At the same time, other researchers point to a case of heartworm disease in a Yorkshire terrier caused by *D. immitis*, where the helminth had a nonspecific localization in the cervical spine. At the same time, no deviations in hematological and biochemi-
ical parameters from the normative ones were found (Bona-
waldt et al., 2017).
Therefore, it is essential to determine the effect of D. immitis helminths on the hematological parameters of infested dogs, considering the intensity of the invasion by microfilariae.

The study aimed to establish changes in hematological parameters of dogs infected with D. immitis, taking into account the intensity of microfilaria invasion

2. Materials and methods

The work was performed during 2018–2021 in the conditions of the private veterinary clinic “Dovira” (Kharkiv) and the laboratory of the Department of Parasitology of the State Biotechnology University.

In order to study the hematological parameters in dogs with heartworm disease, four experimental groups of animals aged 3–10 years weighing 20 to 40 kg (infested with microfilariae and a positive rapid test for D. immitis) were formed, as well as a control group (clinically healthy dogs) for nine goals in each. Depending on the intensity of microfilaria invasion, the first experimental group included dogs with AI less than 20 larvae in 1 cm³ of blood (“+”); in the second group – from II from 20 to 40 larvae/cm³ (“++”); in the third group – from II from 40 to 60 larvae/cm³ (“+++)”; in the fourth group – with II more than 60 lar-

Blood for research was obtained from the superficial vein of the forearm or saphenous vein in the morning before feeding. Determination of hematological parameters was performed according to conventional methods. The number of erythrocytes and leukocytes was counted in Goryaev’s counting chamber; the number of platelets was determined by the method of Fonio; hemoglobin content was determined using a semi-automatic analyzer “BTS-350” (Manufacturer BioSystems, Spain); ESR was determined by the method of Panchenkov; the leukogram was derived by counting leukocytes in blood smears stained with the Leu-
kodif 200 kit (Levchenko et al., 2002; Vlislo, 2012).

A
ll animal manipulations were carried out following the European Convention for the Protection of Vertebrate Ani-
mals Used for Experimental and Scientific Purposes (Stras-
bourg, 1986).

Mathematical analysis of the obtained data was performed using the Microsoft application package “EXCEL” by determining the arithmetic mean (M) and standard error (m).

3. Results and discussion

3.1. Results

Studies have shown that changes in hematological parameters in infected dogs depend on the intensity of the invasion of microfilariae. Thus, the number of erythrocytes in the blood of experimental dogs at the intensity of the invasion “+” was lower by 6.66 % (6.03 ± 0.23 T/L) compared with clinically healthy animals (6.46 ± 0.21 T/L); however, no significant difference between the indicators was found. At the same time, with the intensity of the “++” invasion, the number of erythrocytes in the blood of infected dogs decreased by 11.92 % (5.69 ± 0.19 T/L, P < 0.05) compared with healthy animals. With the increase of AI to “++” and “+++” in the blood of experimental dogs, the number of erythrocytes gradually decreased by 25.54 % (4.81 ± 0.13 T/L, P < 0.001) and 47.21 % (3.41 ± 0.16 T/L, P < 0.001) relative to indicators in clinically healthy animals (Fig. 1).

The content of hemoglobin (142.11 ± 7.17 g/L) and hematocrit (45.89 ± 1.62 %) in the blood of dogs with low intensity of invasion by microfilariae (“+”) compared with similar indicators in clinically healthy dogs, 78 ± 5.08 g/L and 48.11 ± 1.64 %, respectively) had no significant changes. For II “++” changes in the blood of experimental dogs were characterized by a decrease in hemoglobin by 16.33 % (128.67 ± 7.47 g/L, P < 0.05) and hematocrit by 12.70 % (42.00 ± 1.57 %, P < 0.05). Subsequently, for II “+++” and “++++” the above indicators decreased by 25.54 % (4.81 ± 0.13 T/L, P < 0.001) and 47.21 % (3.41 ± 0.16 T/L, P < 0.001) (Figs. 2, 3).

![Fig. 1. Indicators of the number of erythrocytes in the blood of dogs at different intensities of invasion by microfilariae: A – “+”, B – “++”, C – “+++”, D – “++++”, E – “++++”, A – clinically healthy animals](image)

Note: * – P < 0.05, *** – P < 0.001 – relative to indicators in clinically healthy animals

The content of hemoglobin (142.11 ± 7.17 g/L) and hematocrit (45.89 ± 1.62 %) in the blood of dogs with low intensity of invasion by microfilariae (“+”) compared with similar indicators in clinically healthy dogs, 78 ± 5.08 g/L and 48.11 ± 1.64 %, respectively) had no significant changes. For II “++” changes in the blood of experimental dogs were characterized by a decrease in hemoglobin by 16.33 % (128.67 ± 7.47 g/L, P < 0.05) and hematocrit by 12.70 % (42.00 ± 1.57 %, P < 0.05). Subsequently, for II “+++” and “++++” the above indicators decreased by 25.54 % (4.81 ± 0.13 T/L, P < 0.001) and 47.21 % (3.41 ± 0.16 T/L, P < 0.001) (Figs. 2, 3).
**Fig. 2.** Indicators of hemoglobin in the blood of dogs at different intensities of invasion by microfilariae: B – “+”, C – “++”, D – “+++”, E – “++++”, A – clinically healthy animals;

*Note:* * – P < 0.05, *** – P < 0.001 – relative to indicators in clinically healthy animals

**Fig. 3.** Indicators of hematocrit in the blood of dogs at different intensities of invasion by microfilariae: B – “+”, C – “++”, D – “+++”, E – “++++”, A – clinically healthy animals

*Note:* * – P < 0.05, *** – P < 0.001 – relative to indicators in clinically healthy animals

ESR (13.67 ± 1.26 and 16.56 ± 1.56 mm/h) and platelet count (328.33 ± 31.82 and 297.67 ± 27.31 G/L) in dogs with II microfilariae “+” and “++” compared to similar indicators in clinically healthy dogs (12.56 ± 1.42 mm/h and 8.11 ± 0.24 G/L, respectively) had no significant changes. At the same time, for II “+++” and “++++” the gradual acceleration of ESR was established in 1.84 times (23.11 ± 0.75 mm/h, P < 0.001) and 2.51 times (31.56 ± 1.24 mm/year, P < 0.001) (Fig. 4). However, the number of platelets in experimental dogs, on the contrary, gradually decreases by 34.03 % (221.22 ± 13.59 G/L, P < 0.01) and 42.84 % (191.67 ± 5.61 G/L, P < 0.001) (Fig. 5).

The number of leukocytes in the blood of experimental dogs at the intensity of the invasion “+” was lower by 6.78 % (8.70 ± 0.38 G/L) compared with clinically healthy animals (8.11 ± 0.24 G/L); however, no significant difference between the indicators was found. At the same time, at the intensity of the “++” invasion, the number of leukocytes increased by 15.17 % (9.56 ± 0.30 G/L, P < 0.05) in the blood of infected dogs compared with that in clinically healthy animals. With the increase of indicators II to “+++” and “++++” the number of leukocytes also gradually increased by 32.75 % (12.06 ± 0.50 G/L, P < 0.001) and 44.98 % (14.74 ± 0.45 G/L, P < 0.001) (Fig. 6).
Fig. 4. Indicators of ESR in the blood of dogs at different intensities of invasion by microfilariae:

Note: *** – P < 0.001 – relative to indicators in clinically healthy animals

Fig. 5. Indicators of the number of platelets in the blood of dogs at different intensities of invasion by microfilariae:

Note: ** – P < 0.01, *** – P < 0.001 – relative to indicators in clinically healthy animals

Fig. 6. Indicators of the number of leukocytes in the blood of dogs at different intensities of invasion by microfilariae:

Note: ** – P < 0.01, *** – P < 0.001 – relative to indicators in clinically healthy animals
Analyzing the indicators of leukoformula in dogs with parasitism Dirofilaria immitis, it can be noted that with the increase of II microfilariae significantly increases the percentage of eosinophils: for “+” – 1.83 times (5.89 ± 0.93 %, P < 0.001), for “+++” – 2.14 times (6.89 ± 0.35 %, P < 0.001), for “++++” – 2.66 times (8.56 ± 0.29 %, P < 0.001) compared with clinically healthy animals (3.22 ± 0.32 %) (Table 1).

Table 1
Indicators of the leukogram of dogs with heartworm invasion, % (M ± m, n = 9)

<table>
<thead>
<tr>
<th>Indicators</th>
<th>–</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Clinically healthy animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4.00 ± 0.24</td>
<td>5.89 ± 0.93***</td>
<td>6.89 ± 0.35***</td>
<td>8.56 ± 0.29***</td>
<td>3.22 ± 0.32</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>20.56 ± 0.88</td>
<td>18.11 ± 0.82**</td>
<td>15.33 ± 0.80***</td>
<td>12.00 ± 0.58***</td>
<td>22.00 ± 1.03</td>
</tr>
<tr>
<td>Monocytes</td>
<td>5.78 ± 0.15</td>
<td>5.78 ± 0.67</td>
<td>6.89 ± 0.42</td>
<td>7.67 ± 0.47**</td>
<td>5.89 ± 0.26</td>
</tr>
</tbody>
</table>

In addition, the II “++++” in experimental dogs showed an increase in the number of rod neutrophils by 1.38 times (4.44 ± 0.41 %, P < 0.05) and monocytes by 1.30 times (7.67 ± 0.47 %, P < 0.01) relative to indicators in clinically healthy animals (3.22 ± 0.32 and 5.89 ± 0.26 %, respectively). The number of lymphocytes in the blood of experimental dogs with increasing indicators of AI gradually decreased: for “+” – 1.21 times (18.11 ± 0.82 %, P < 0.01), for “++” – 1.44 times (15.33 ± 0.80 %, P < 0.01), for “+++” v 1.83 times (12.00 ± 0.58 %, P < 0.001) compared with clinically healthy animals (22.00 ± 1.03 %).

3.2. Discussions
Since scientists have proven significant pathogenicity of nematodes of the species Dirofilaria immitis Leidy, 1856, it is important to establish various criteria that determine the severity of the disease in animals, including hematological parameters (Donahoe et al., 1976; Maerz, 2020; Romano et al., 2021). Our research has shown that the severity of deviations from the hematological parameters of dogs with heartworm disease caused by parasitism of D. immitis depends on the level of microfilaremia. In particular, with the increase in the intensity of microfilaremia infestation in infected dogs, the number of erythrocytes gradually decreased (by 11.92–47.21 %, P < 0.05… P < 0.001), platelets (by 34.03–42.84 %, P < 0.01… P < 0.001), hemoglobin content (by 16.33–42.27 %, P < 0.001), hematocrit (by 12.70–41.57 %, P < 0.001). At the same time, the number of leukocytes gradually increased (by 15.17–44.98 %, P < 0.05… P < 0.001), and ESR acceleration was noted (1.84–2.51 times, P < 0.001). In our opinion, the development of anemia and leukocytosis in dogs infected with heartworms is due to the mechanical effects of parasites and products of their metabolism on animals. As a result, circulating blood cells are destroyed, and inflammatory phenomena occur in the organs and tissues of dogs. The studies revealed changes in the leukoformula of experimental dogs, which were characterized by an increase in the number of eosinophils (1.83–2.66 times, P < 0.001), rod-shaped neutrophils (1.38 times, P < 0.05), monocytes (1, 30 times, P < 0.01) and a decrease in the number of lymphocytes (1.21–1.83 times, P < 0.01… P < 0.001). Similar data were obtained by researchers who note that hematological changes in cardiac heartworm disease depend on the severity of the invasion and the intensity of the invasion of microfilariae. Thus, in the early stages of invasion with low rates of invasion intensity, the authors observed eosinophilia, and in the latter stages with high rates of invasion - anemia, decreased hemoglobin, hematocrit, as well as leukocytosis, neutrophilia, eosinophilia, monocytosis (Harvey et al., 1982; Goggin et al., 1997; Sevimli et al., 2007).

Therefore, the results of the studies indicate the importance of determining hematological parameters in heartworm disease, which complements the picture of clinical examination of animals and will allow more effective treatment of dogs in this invasion.

4. Conclusions
It was found that parasitism of nematodes of the species Dirofilaria immitis leads to changes in hematological parameters of infected dogs, depending on the intensity of the invasion by microfilariae. In the blood of animals for parasitism, 20 larvae/cm³ deviations from physiological parameters were not observed. Indicators of the intensity of invasion of 20–40 larvae/cm³ showed signs of anemia, and inflammatory and allergic phenomena, as evidenced by a decrease in the number of erythrocytes, hemoglobin, hematocrit, increase in the number of leukocytes, eosinophils. At the same time, with the intensity of the invasion of 40–60 larvae/cm³ and more than 60 larvae/cm³, the phenomena of anemia, leukocytosis, and eosinophilia in the blood of infected dogs increase, and ESR acceleration, thrombocytopenia, lymphocytopenia, and neutrophils are observed.

Conflict of interest.
The author state that there is no conflict of interest.

References

Ukrainian Journal of Veterinary and Agricultural Sciences, 2022, Vol. 5, N 1
Dantas-Torres, F., & Otranto, D. (2013). Dirofilariosis in the Americas: a more virulent Dirofilaria immitis? Parasites & Vectors, 6(1), 288. [Crossref] [Google Scholar]


Genchi, C., & Kramer, L. H. (2020). The prevalence of Dirofilaria immitis and D. repens in the Old World. Veterinary Parasitology, 280, 108995. [Crossref] [Google Scholar]

Genchi, M., Rinaldi, L., Venco, L., Cringoli, G., Vismarra, A., & Kramer, L. (2019). Dirofilaria immitis and D. repens in dog and cat: A questionnaire study in Italy. Veterinary Parasitology, 267, 26–31. [Crossref] [Google Scholar]


Harvey, J. W., Henderson, C. W., French, T. W., & Meyer, D. J. (1982). Myeloproliferative disease with megakaryocytic predominance in a dog with occult dirofilariasis. Veterinary Clinical Pathology, 11(1), 5–11. [Crossref] [Google Scholar]


Jiménez-Penago, G., Hernández-Mendo, O., González-Garduño, R., Torres-Hernández, G., Torres-Chablé, O. M., & Maldonado-Simán, E. (2021). Mean corpuscular haemoglobin concentration as haematological marker to detect changes in red blood cells in sheep infected with Haemonchus contortus. Veterinary Research Communications, 45(4), 189–197. [Crossref] [Google Scholar]


Maerz, I. (2020). Clinical and diagnostic imaging findings in 37 rescued dogs with heartworm disease in Germany. Veterinary Parasitology, 283, 109156. [Crossref] [Google Scholar]


Sevimli, F. K., Kozan, E., Bulbül, A., Birdane, F. M., Köse, M., & Sevimli, A. (2007). Dirofilaria immitis infection in dogs: unusually located and unusual findings. Parasitology Research, 101(6), 1487–1494. [Crossref] [Google Scholar]


Younes, L., Barré-Cardi, H., Bedjaoui, S., Ayhan, N., Varlound, M., Mediannikov, O., Otranto, D., & Davoust, B. (2021). Dirofilaria immitis and Dirofilaria repens in mosquitoes from Corsica Island, France. Parasites & Vectors, 14(1), 427. [Crossref] [Google Scholar]