

Efficacy of ultrasound in diagnosis of dirofilariasis in dogs caused by *Dirofilaria immitis*

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Ultrasonic diagnostics is a modern and effective method of laboratory testing of both animals and humans, used for estimation of overall state, and for detection of different pathologies in organs and systems. The aim of the present study was to establish the efficacy of ultrasonic diagnostics of dog dirofilariasis caused by *Dirofilaria immitis* parasitism, based on several indexes of invasion intensity. It was established that the number of *D. immitis* helminths found depended on the intensity indexes of microdироfilariasis invasion: using echocardiography, there were no helminths found under the index lower than 40 larvae/cm³. Under the index higher than that, we visualized 1–10 parasite individuals in the cavity of the right ventricle and right atrium, and at the tricuspid valve leaflets. It was established by the results of echocardiography of dogs invaded by *D. immitis* that the level of invasion intensity impacts significantly on the animals' heart structure and function, and on their intracardiac hemodynamics. It was proved that under increase of microfilaria number in the blood, heart function deteriorates. Under the invasion intensity lower than 40 larvae/cm³, the changes were characterized by a slight increase in thickness of the posterior wall of the left ventricle in diastole, aorta diameter, left atrium and pulmonary artery (by 6.2%). Under the invasion intensity of 40–60 larvae/cm³, the deterioration in heart structure and function was followed by thickening of the interventricular septum in diastole, posterior wall of the left ventricle in diastole (by 5.6%), increase in ejection fraction index (by 7.0%), widening of aorta root, pulmonary artery, diameter of the left ventricle (by 21.4%), and with increase in indexes of ratio between diameters of the left ventricle and pulmonary artery, and aorta (by 15.6%). Signs of hemodynamic deterioration were established in sick animals, which was characterized by increase of blood speed in the tricuspid valve (by 33.7%), and its decrease in the pulmonary artery (14.1%). Under the invasion intensity higher than 60 larvae/cm³, the aforementioned indexes changed even more significantly. We observed thickening of the posterior wall of the right ventricle in diastole, decrease of the end-systolic size of the left ventricle (by 13.0%), blood speed increase in the aorta and mitral valve (by 12.2%). The obtained results show the necessity of echocardiographic testing of dogs with cardiac dirofilariasis, which allows effective proof of the diagnosis and also helps to understand the overall state of the animal more deeply and to provide it with the appropriate symptomatic cure.

Keywords: zoonanthropotic disease; nematodes; microfilariae; invasion intensity; echocardiography; informativeness.

Introduction

The basis of dirofilariasis diagnostics consists of complex analysis of epizootiological data, clinical symptoms, pathoanatomic changes, and results of laboratory tests (Strickland, 1998; Hoch & Strickland, 2008; Romano et al., 2021; Yemolenko et al., 2022). In particular, hemolarvascopy is used, which is based on blood testing via several methods of different efficacy, which can be ineffective in case of presence of immature nematodes in the organism of the definitive host (Magnis et al., 2013; Ionica et al., 2017; Genchi et al., 2021). Also, the industry offers plenty of convenient immuno-chromatographic diagnostic express-tests. Genetic methods are used as well. They are more sensitive, aimed at the detection of both mature and immature *Dirofilaria*, and allow the species of parasite to be identified (Albonico et al., 2014; Borthakur et al., 2015). Scientists note the importance of additional instrumental methods, such as roentgenology and echocardiology (Venco et al., 1996; Little et al., 2018; Corda et al., 2022).

Despite the widespread nature of dirofilariasis, caused by *D. immitis*, among the wild carnivores, morphofunctional research on the effect of this invasion on cardio-vascular anatomy, lung and other systems has been insufficiently emphasized in clinical veterinary practice. Nowadays, there are no clear criteria for the course of cardiac dirofilariasis in relation to invasion intensity indexes, using such an instrumental method as echocardiography. In particular, in cases of high invasion intensity, when parasites

localize in the lung arteries, right ventricle and right atrium, scientists have observed the widening or hypertrophy of the right ventricle, paradoxical interventricular septal motion, pericardial effusion, tricuspid valve regurgitation, blood pressure increase in the lung arteries, and narrowing or thickening of septum (Browne et al., 2005; Oldach et al., 2018; Saunders et al., 2020).

The authors have stated that the hypertrophy of right ventricle and the deterioration of its diastolic functions are the early criterion of compensated stage, which appears due to dirofilariasis parasitism. The decompensated stage is characterized by significant dilatation with decreased stroke volume and cardiac output, and the deteriorated systolic function. According to their results, animals with preclinical dirofilariasis develop right ventricle hypertrophy, which is caused by long-term obstruction of the blood outflow due to obstruction of the lung arteries by helminths. Further, the syndrome of chronic pulmonary heart disease emerges when hypertrophy of the right ventricle wall and interventricular septum develop (Kitagawa et al., 1990; McCracken & Patton, 1993; Kelliham & Stepien, 2010). Also, scientists report that the exhaustion of compensatory mechanisms leads to emergence of right-ventricle insufficiency symptoms, and clinical manifestations of cardio-dirofilariasis. The emerged pathology is complicated due to widening of the atrioventricular canal. This is followed by tricuspid valve insufficiency and further right atrium dilatation. The development of these processes is followed by expressed insufficien-

cy in systemic circulation and correlates with further widening of the right heart. As the result, the significant increase of afterload and decrease of preload of the left ventricle are observed, which lead to decrease of its systolic function indexes (Atkins et al., 1988; Venco et al., 2014; Falcón-Cordón et al., 2019).

Thus, considering that *D. immitis* is the most pathogenic animal parasite species, provoking severe illness with a specific course (absence of visible clinical traits at the beginning, their non-specificity, different localization of parasites at different developmental stages), the effective detection of parasites would help to prevent complications in the cardiovascular and lung systems. That is why the aim of the present work was to establish the efficacy of ultrasonic diagnostics of dirofilariasis in dogs subject to *D. immitis* parasitism using different indexes of microfilariae invasion intensity indexes.

Materials and methods

The research was performed during 2018–2022 in the private veterinary clinic “Dovira” (Kharkiv, Ukraine), in the Parasitology Laboratory of the State Biotechnology University, and in the Parasitology and Veterinary-Sanitary Expertise Department of Poltava State Agricultural University. The research protocol of the current study was approved by the Ethics Committee of Poltava State Agrarian University. All animal manipulations were carried out following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986).

The ultrasonic study of the dogs’ hearts was carried out using Chison Qbit 5 (Chison, China) with: phase sensors 3.5–5.6 MHz, sensor micro-convection 6.5–11.7 MHz. The skin site where sensors were placed was shaved and treated with 70%-ethanol, and then gel for the ultrasonic test was applied.

To establish the efficacy of ultrasonic diagnostics of cardiac dirofilariasis, we formed four groups of experimental dogs of 3–10 years age, 20–40 kg mass, and of different breeds: mongrels, German Shepherd, Rottweiler, Labrador Retriever, Caucasian Shepherd, Alaskan Malamute. The experimental groups were formed by the dogs invaded by microfilariae with different invasion intensity with positive *D. immitis* express-tests

(CaniV-4, Vet Expert, Poland), and the control group was formed by the clinically healthy dogs; each group contained 9 dogs. Depending on the invasion intensity, the first experimental group contained dogs with intensity of less than 20 larvae/cm³ (“+”), the second – 20–40 larvae/cm³ (“++”), third – 40–60 larvae/cm³ (“+++”), fourth – more than 60 larvae/cm³ (“++++”).

We performed the echocardiographic estimation of thickness of the interventricular septum in diastole and systole (IVSd and IVSs, mm), thickness of the left ventricle posterior wall in diastole and systole (mm), end-diastolic and end-systolic size of the left ventricle (mm), thickness of the right ventricle posterior wall in diastole (mm), aorta diameter (mm), left atrium diameter (mm), pulmonary artery diameter (mm), ratio between diameters of the left atrium and aorta, ratio between diameters of the lung artery and aorta, ejection fraction of the left ventricle (%), mitral valve blood speed (m/s), tricuspid valve blood speed (m/s), aorta blood speed (m/s), pulmonary artery blood speed (m/s).

Statistical analysis of the experimental results was carried out using Statistica 10 (StatSoft Inc., USA) software. Mean values (\bar{x}) and standard deviations (SD) were calculated. Significance of difference between average values of the experimental and control groups was established using one-way analysis of variance and F-test (with consideration of Boniferroni’s correction) for $P < 0.05$ level.

Results

The results of the performed instrumental studies of experimental groups of dogs showed that the number of detected helminths *D. immitis* depends on the indexes of microfilariae invasion intensity. In particular, under the invasion of less than 40 larvae/cm³ (“+”, “++”), there were no adult dirofilariasis found using echocardiography. Under the “+++” invasion, the UT detected 1–5 individuals, and under the “++++” invasion 6–10 individuals; *D. immitis* were visualized in the cavity of the right ventricle, right atrium, and at the tricuspid valve leaflets (Fig. 1).

The analysis of echocardiography-derived indexes showed the significant impact of invasion intensity level on the state of dogs’ cardiovascular system: the higher the index, the more significant changes occur in their organisms (Fig. 2).

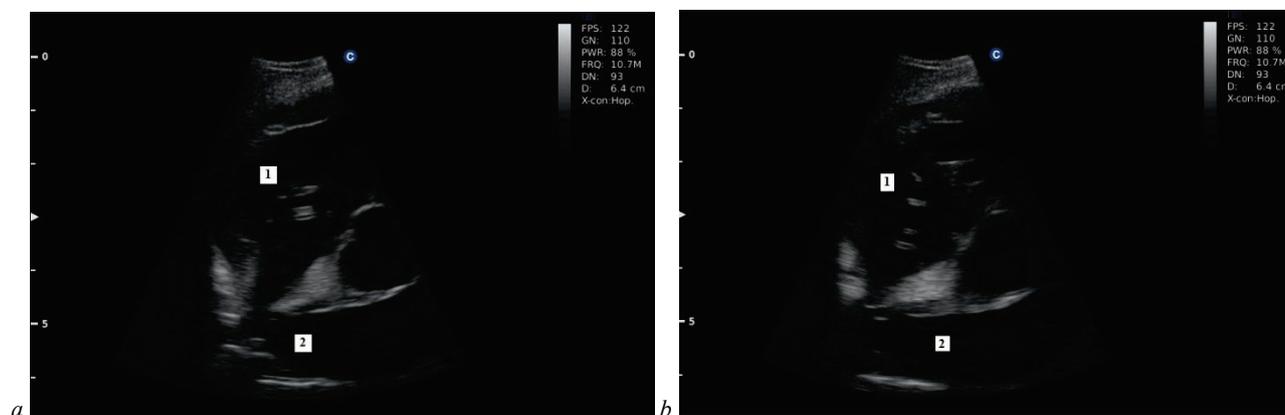


Fig. 1. The results of dog’s heart echocardiography: *a* – right-sided parasternal short-axis projection at the level of pulmonary trunk in the ventricle’s diastole phase; *b* – right-sided parasternal short-axis projection at the level of pulmonary trunk in the ventricle’s systole phase; 1 – visualization of *Dirofilaria immitis* in the cavity of right ventricle, right atrium, and at the tricuspid valve leaflets; 2 – widening of right branch of lung artery; FPS – frames per second, GN – gain setting, PWR – ultrasound power/wattmeter; FRQ – frequency used from the ultrasound transducer, DN – digital number, D – depth markers

At the “+” invasion level, the experimental animals had increase in thickness of the posterior wall of the left ventricle in diastole by 3.4% ($P < 0.0125$, 8.57 ± 0.23 mm) compared to healthy animals. At the “++” invasion, the sick animals had the increase in thickness of the posterior wall of the left ventricle in diastole by 4.4% ($P < 0.0125$, 8.66 ± 0.34 mm, Fig. 2c). At the “+++” invasion, the changes in heart structure were characterized by increase in thickness of the interventricular septum in diastole by 3.4% ($P < 0.0025$, 8.72 ± 0.14 mm), and increase in thickness of the posterior wall of the left ventricle in diastole by 5.6% ($P < 0.0025$, 8.77 ± 0.33 mm, Fig. 2a–c). At the high invasion intensity (“++++”), the heart structure changes were the most significant: thickness of the inter-

ventricular septum in diastole and systole increased by 5.4% ($P < 0.00025$, 8.90 ± 0.08 mm) and 3.5% ($P < 0.0125$, 13.03 ± 0.18 mm), respectively, thickness of the posterior wall of the left and right ventricle in diastole increased by 5.9% ($P < 0.00025$, 8.80 ± 0.08 mm) and 3.8% ($P < 0.025$, 9.17 ± 0.13 mm), and the end-systolic size of the left ventricle decreased by 13.0% ($P < 0.00025$, 19.13 ± 0.56 mm, Fig. 2a–c, e, g).

Also, the “+” and “++” invasions (Fig. 3) were followed by significant ($P < 0.00025$) widening of aorta root diameter by 5.6% (19.66 ± 0.19 mm) and 5.7% (19.69 ± 0.21 mm), respectively (Fig. 3a), left atrium diameter by 4.5% (23.64 ± 0.41 mm) and 5.8% (23.96 ± 0.30 mm), respectively (Fig. 3c), and lung artery diameter by 6.2% (20.54 ± 0.21 mm)

and 7.7% (20.88 ± 1.11 mm), respectively (Fig. 3b). At high indexes of invasion intensity (“+++”, “++++”), the aorta root of experimental dogs widened by 7.5% and 8.2% ($P < 0.00025$ – to 20.22 ± 0.32 mm, Fig. 3a), the lung artery by 21.4% and 31.4% ($P < 0.00025$ – to 28.07 ± 0.22 mm, Fig. 3b), and the left atrium by 11.4% and 17.7% ($P < 0.00025$ – to 27.43 ± 0.05 mm, Fig. 3c). We also detected increased diameter ratio between the left atrium and aorta by 4.7% ($P < 0.0125$, 1.27 ± 0.04) and

11.0% ($P < 0.00025$, 1.36 ± 0.03 , Fig. 3c), and the pulmonary artery and aorta by 15.6% and 25.9% ($P < 0.00025$ – to 1.39 ± 0.02 , Fig. 3e). The tests on heart work efficiency showed that the ejection fraction index of the left ventricle increased only under the significant (“+++” and “++++”) invasions by 7.0% and 12.3% ($P < 0.00025$ – to $74.50 \pm 3.03\%$, Fig. 3f).
The higher the changes of intracardiac hemodynamics, the higher invasion intensity index (Fig. 4).

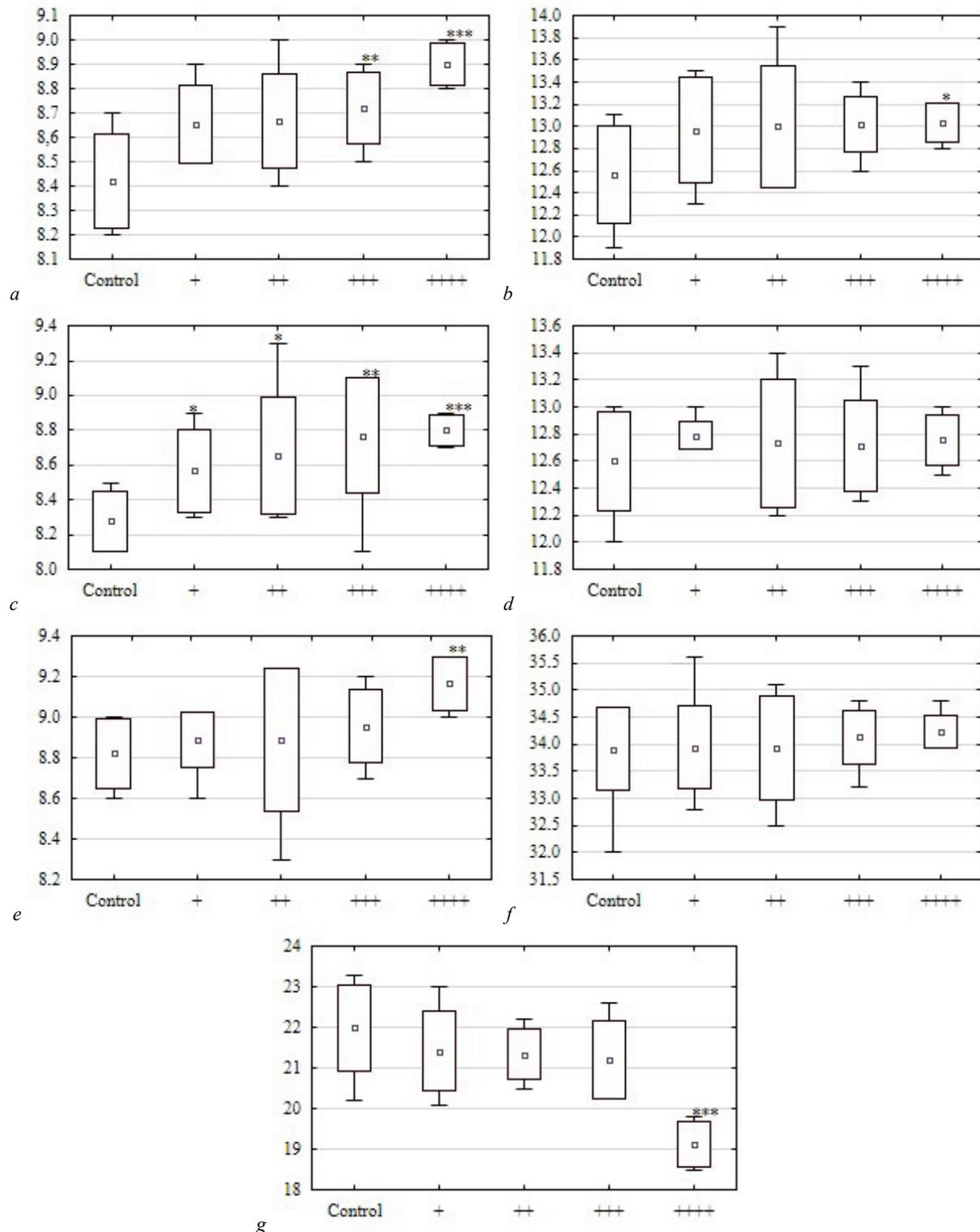


Fig. 2. The dynamics of change for indexes of heart structure and function by the results of echocardiography dependent on the level on microfilariae invasion intensity; thickness of the interventricular septum in diastole (a) and systole (b), thickness of left ventricle posterior wall in diastole (c) and systole (d), thickness of right ventricle posterior wall in diastole (e), end-diastolic (f) and end-systolic (g) size of left ventricle; the small square in the centre corresponds to the mean values (\bar{x}), the lower and upper borders of the large rectangle correspond $\bar{x} \pm SD$, vertical line segments, directed up and down from the rectangle, correspond to minimum and maximum values ($n=9$); * – $P < 0.0125$, ** – $P < 0.0025$, *** – $P < 0.00025$ – compared to the dogs from the control group (with Bonferroni correction)

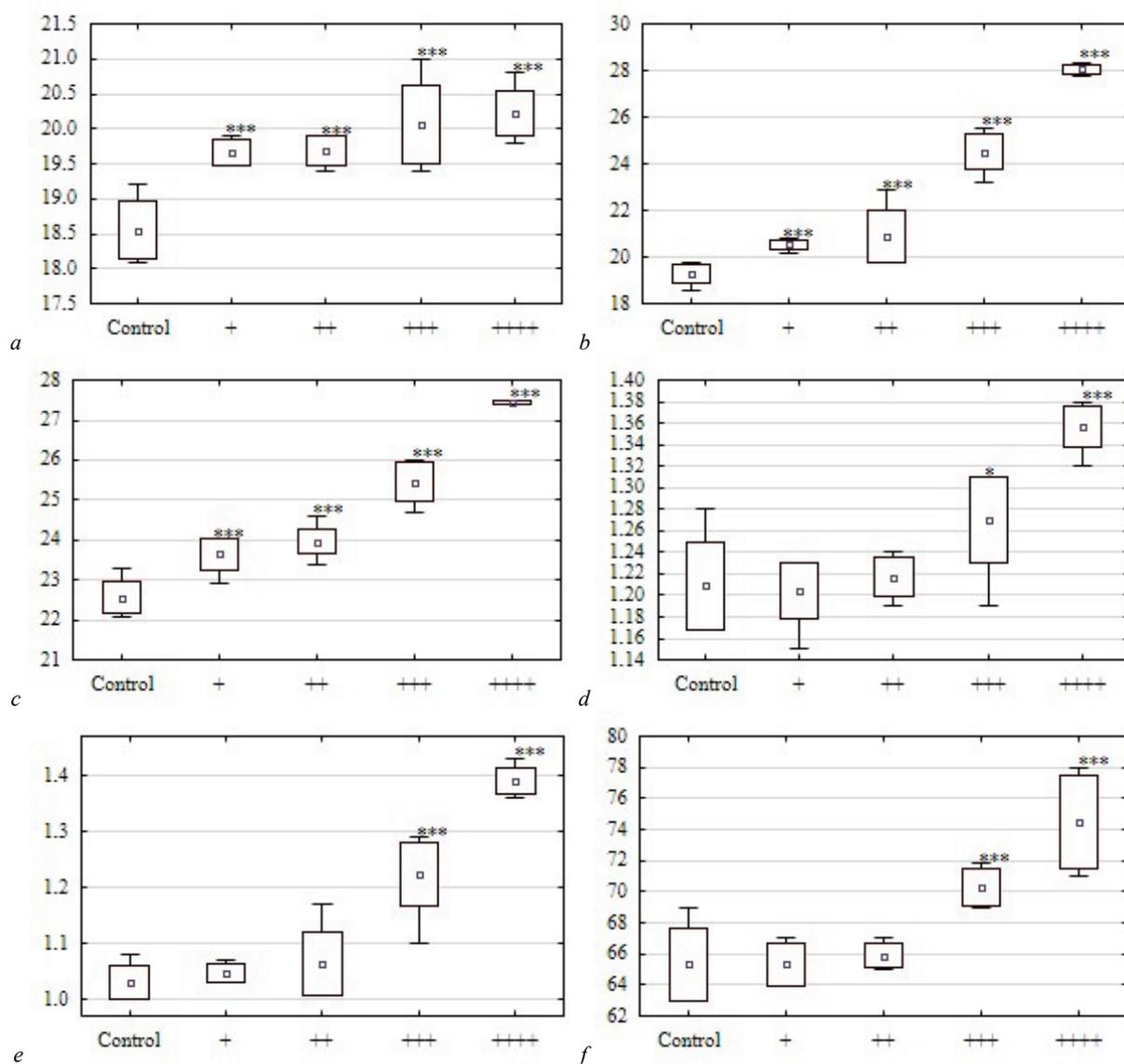


Fig. 3. The dynamics of diameter changes of aorta (a), pulmonary artery (b), left atrium (c), the ratio between diameters of left atrium and aorta (d), and between diameters of pulmonary artery and aorta (e), ejection fraction of left ventricle (f) by the echocardiography results dependent on the invasion intensity level; see Figure 2

So, at the “+” and “++” intensity, there were no significant changes in either experimental or control groups. At the “+++” invasion, the blood speed increased in the tricuspid valve by 33.7% ($P < 0.0125$, 0.98 ± 0.03 m/s), and decreased in the pulmonary artery by 14.1% ($P < 0.0025$, 0.85 ± 0.05 m/s, Fig. 4b, d). At the “++++” invasion, the changes were the most significant: blood speed increased in the mitral valve by 12.2% ($P < 0.0125$, 0.74 ± 0.05 m/s), increased in the tricuspid valve by 51.5% ($P < 0.001$, 1.34 ± 0.02 m/s), increased in the aorta by 5.6% ($P < 0.0125$, 1.25 ± 0.05 m/s), and decreased in the pulmonary artery by 25.3% ($P < 0.0125$, 0.74 ± 0.05 m/s, Fig. 4a–d).

Discussion

According to the literature data, a number of dirofilariæ species have been detected and described in dogs and other carnivores, among which the most widespread and pathogenic is *Dirofilaria immitis* Leidy, 1856. It is associated with the localization of these parasites in the right ventricle and lung arteries, which causes severe deteriorations of all systems in the organism, especially the cardiovascular, and may cause the animal’s death (Sim et al., 2013; Maerz, 2020; Romano et al., 2021). That’s why the timely and effective detection of these parasites using the ultrasonic diagnostics, along with establishing the entire state of the organism is important and allows complications in these systems to be prevented.

The results of echocardiography showed that no helminths were found using ultrasonic diagnostics at the invasion intensity of lower than 40 larvae/cm³. At the same time, at the invasion intensity of 40–60 larvae/cm³, we found 1–5 parasites in cavities of the right ventricle, right atrium, and at the tricuspid valve leaflets, and at more than 60 larvae/cm³ intensity – 6–10 parasites. Some authors have noted such a dependence of dirofilariæ detection, when microfilariae were known to be present in the blood but were not detected. According to them, the presence of *D. immitis* in the heart shows a high level of invasion. It is caused by the primary localization of mature helminths in the lung arteries, whence they move to the right heart when their number increases to over 50 nematodes (Feshchenko et al., 2020).

Echocardiography also showed that invasion level influences the heart structure and function, and intracardiac hemodynamics of sick animals. Particularly, at the invasion intensity lower than 40 larvae/cm³, the changes consisted of thickening of the posterior wall of the left ventricle by 4.4% ($P < 0.0125$) in diastole, aorta diameter by 5.7%, left atrium by 5.8%, and lung artery by 7.7% ($P < 0.00025$). Such changes indicate reactive thickening of the endocardium and development of arterial hypertension. Due to increase in pressure in the lung artery stem, the systolic function of the right ventricle decreases. Similar data is described by other authors, stating the complex connection between presence of helminths in the heart and development of lung hypertension (Kitagawa et al., 1990).

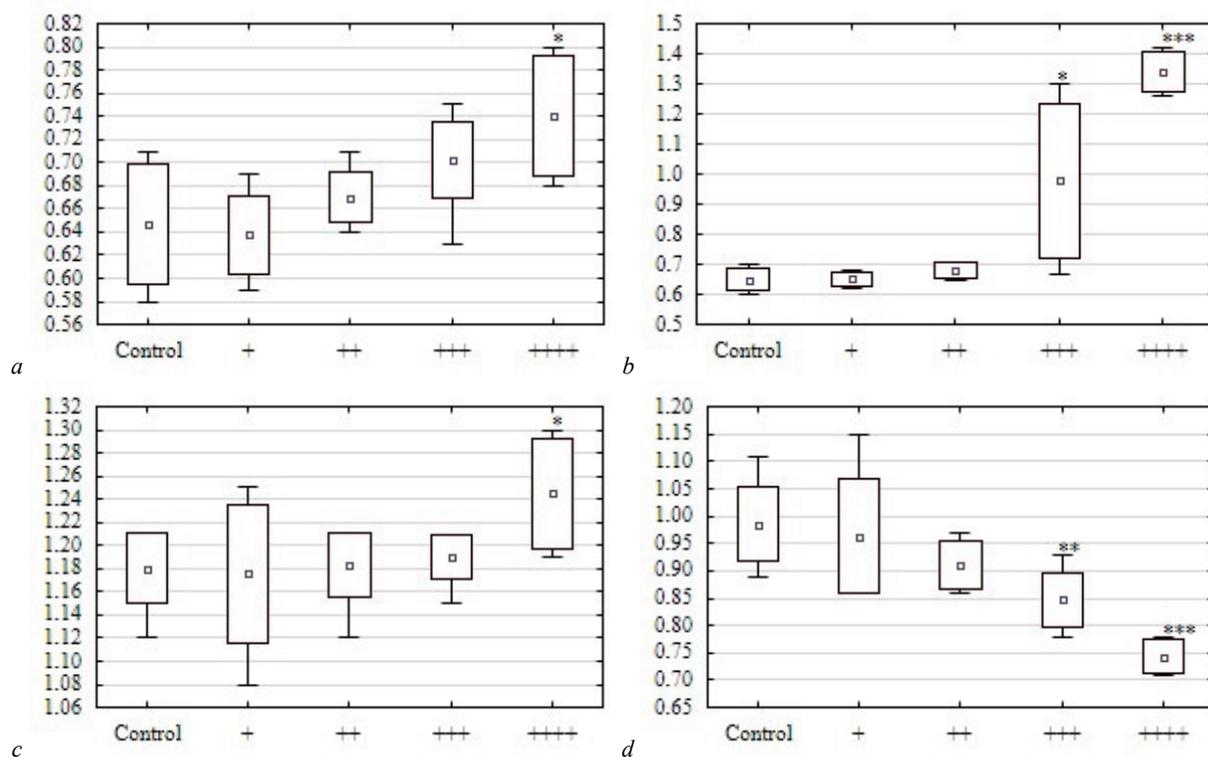


Fig. 4. The dynamics of intracardiac hemodynamics changes by the results of echocardiography of dogs dependent on the invasion intensity level: mitral valve blood speed (a), tricuspid valve blood speed (b), aorta blood speed (c), pulmonary artery blood speed (d); see Figure 2

At the invasion 40–60 larvae/cm³, the heart structure and function deterioration was followed by increasing signs of lung hypertension. Also, the signs of intracardiac hemodynamic deterioration were established, which was characterized by blood speed increasing in the tricuspid valves by 33.7% ($P < 0.0125$), decreasing in the pulmonary artery by 14.1% ($P < 0.0025$), and increase in ejection fraction of left ventricle by 7.0% ($P < 0.00025$). Such a change may indicate the partial thromboembolism caused by dirofilariasis, as well as progression of invasion in accordance with the parasite's life cycle (Slonka et al., 1977; Lee et al., 2010).

With the invasion intensity higher than 60 larvae/cm³, the aforementioned indexes changed even more significantly in the invaded dogs. There were simultaneously detected thickening of the posterior wall of the right ventricle in diastole by 3.8% ($P < 0.0025$), decrease in end-systolic size of the left ventricle in diastole by 13.0% ($P < 0.00025$), and increase in blood speed in the aorta by 5.6% ($P < 0.0125$). Such changes are thought by scientists to be the signs of beginning of compensatory hypertrophy of the left ventricle, tonogenic widening of the aorta canals and pulmonary artery, and increase in the left atrium diameter. Retrograde migration of mature dirofilariae from the pulmonary artery to the right ventricle, right atrium and venae cavae provokes the problems with the tricuspid apparatus. Valve insufficiency along with lung hypertension leads to heart failure. Under significant invasion, decompensatory phenomena are observed, when dilatation of right ventricle and right atrium occurs. Besides, the blood composition changes, and its density rises, due to the presence of microfilariae in large numbers (Strickland, 1998; Bove et al., 2010).

The obtained results show the necessity of echocardiographic testing of dogs with cardiac dirofilariasis, which allows the diagnosis to be proved effectively and also helps to understand the overall state of the animal more deeply and to provide it with the appropriate symptomatic cure.

Conclusion

The conducted echocardiographic tests on the dogs invaded by *Dirofilaria immitis* Leidy, 1856 showed the nematodes in the cavities of right ventricle, right atrium and at the tricuspid valve leaflets, only under the indexes of microfilariae invasion intensity higher than 40 larvae/cm³. It was established that along with increase in invasion intensity indexes,

changes in the heart structure increased as well, with deterioration in its function and intracardiac hemodynamics. In dogs with dirofilariasis of the heart, thromboembolism of the aorta and pulmonary artery emerges, function of the valve apparatus worsens, lung hypertension and heart failure develop, and blood density rises, as the result of microfilariae migration. Echocardiographic testing at high invasion intensity proved this by showing increased thickness of the interventricular septum by 5.4% and of the posterior walls of left and right ventricles in diastole by 5.9% and 3.8%, respectively, widening of the diameter of aorta by 8.2%, pulmonary artery by 31.4% and left atrium by 17.7%, increase in the diameter ratio between the left atrium/ pulmonary artery and aorta to 11.0% and 25.9%, respectively, increase in ejection fraction by 12.3%, increase in blood speed in the mitral and tricuspid valves and in aorta by 12.2%, 51.5% and 5.6%, respectively, and decrease in blood speed in the pulmonary artery by 25.3%.

The authors state that there is no conflict of interest.

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