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VECTOR BORN DISEASES IN DOGS – DIROFILARIOSIS AND ANAPLASMOSIS. A CLINICAL STUDY

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ABSTRACT

Canine vector-borne diseases are an important group of illnesses affecting dogs around the world. The transmission of these diseases to the dogs is through different arthropod vectors, such as ticks, fleas, lice, triatomines, mosquitoes, tabanids, and phlebotomine sand flies.

For the period of years 2014–2016 we made 160 tests for these diseases. Twelve of these animals were positive for Dirofilaria immitis, 14 were positive for Anaplasmosis and 10 of them were positive for Ehrlichiosis. There were no positive tests for Lime disease. Part of these animals was treated through a specific therapy according to their condition.

Key words: vector-born diseases, dog, dirofilariosis, anaplasmosis.

INTRODUCTION

Heartworm

The dirofilariosis (Heartworm) is a parasitic vector transmitted disease, caused by the nematode Dirofilaria immitis. This parasite infects mainly dogs and cats but also wild animals and other carnivores including humans could be affected too. The adult parasites are localized in the pulmonary arteries and the right ventricle of the heart, accompanied by clinical manifestation known as Canine Heartworm disease (HWD). In some cases, it may be established abnormal migration with an ectopic location – body cavities, central nervous system, eyes, etc.

The biological cycle of the parasite consists of five larval stages. In the intermediate hosts the embryo develops to invasion capable L3 larva. After inoculation, in the final host the L3¬ larva develops into an adult individual. The prepatent period is 120 to 180 days. Sexually matured individuals emit microfilariae in the blood of the final hosts and intermediate hosts are being infected when sucking blood from microfilaric hosts (McCall J. et al., 2008). The invasion capable larvae are transmitted from several mosquitoes, including – genus Culex, Aedes, Ochlerotatus, Anopheles, Armigeres and Mansoria, although Aedes vexans, Culex pipiens and Aedes albopictus are the main natural vectors of the filaric worms in Europe (Cancrini G. et al., 2006, Cancrini G. et al., 2007).

Transmission of the dirofilaria depends of several basic factors: enough number of microfilaric dogs, which serve as a reservoir of infection; suitable mosquitoes; suitable climate to allow incubation in the intermediate hosts (Genchi C. et al., 2005).

A necessary condition for reproduction of adult heartworms is Gram-negative bacteria of the genus Wolbachia, which is an obligate endosymbiont of Dirofilaria immitis. Wolbachia sp. is essential for the survival and reproduction of most pathogenic filaric worms, it is transmitted vertically and worm prevalence is 100 % (Kramer L. et al., 2005).

Many other factors play a critical role in the vector capacity of mosquitoes, including genetic characteristics, the morphology of their cibarial membrane which is able to reduce the number of

ingested microfilariae during the blood sucking (Lori A. et al., 1990), protozoan infections, availability of nutrients in the environment, etc. (Comiskey N. et al., 1990). Passing of the pets through borders increases the risk of distribution of filaric infections as well as other vector infections (Genchi C. et al., 2011; Pantchev N. et al., 2011).

Anaplasmosis

Anaplasmosis is a vector zoonosis, which can occur in two forms – trombocytic and granulocytic. The causatives are Rickettsia microorganisms belonging to the family Anaplasmataceae.

Trombocytic – the infectious canine cyclic thrombocytopenia, also known as canine thrombocytic Anaplasmosis is caused by Anaplasma platys, which is Gram-negative, intracellular, obligate bacteria that infects canine thrombocytes.

Anaplasma platys, known earlier as Ehlichia platys, is found and described for the first time in 1978 in peripheral thrombocytes of dogs from Florida (Harvey J. et al., 1978). After infection the typical morules in the thrombocytes of the peripheral blood develop from 10 to 14 days, followed by thrombocytopenia in the period 14-21 days after infection (Gaunt S. et al., 1990).

The thrombocytopenia and the parasitemia appear cyclically at approximately 10-14 days intervals (Harvey J.et al., 1978). It has been found that the severity of the thrombocytopenia and the percent of invaded platelets reach their maximum during the first phase of the disease. Intensification of thrombocytopenia is not associated with the increase of the parasitemia. Peripheral thrombocytopenia is accompanied by an increase in the number of bone marrow megakaryocytes – regenerative thrombocytopenia (Gaunt S. et al., 1990).

Granulocytic anaplasmosis – Anaplasma phagocytophilum is Gram-negative, immobile, obligate intracellular bacteria of the family Anaplasmataceae (Dumler J. et al., 2001). In mammals it infects the granulocytes – mainly neutrophils, but it could be found in eosinophils as well (Klein M. et al., 1997; Mutnderloh U. et al., 2004).

After staining with Romanovsky – Giemsa, the anaplasmas, which are morules in cytoplasmic vacuoles, are visualized as round bodies with bluish-purple inclusions inside with a diameter between 1.5 to 6 micrometers (Goodman J. et al., 1978).

Studies show that anaplasmas are transmitted after bites from ticks of the genus Ixodes, as the main factor for Europe is I. ricinus.

Anaplasmas could be identified among other genera ticks as well - Haemaphusalis punctate (Macleod J., 1962), Rhipicephalus sanguineus (Albert A. et al., 2005), etc., although not yet proven as effective vectors.

Within the vector Anaplasmosis is transmitted transstadially and could survive in infected tick for years until it infects new host; vertical transmission is inefficient (Ogden N. et al., 2002). Anaplasmosis infection is performed approximately 36-48 hours after the tick bite.

The purpose of this clinical study was to determine the effectiveness of specific treatments applied in animals suffering from heartworm disease and anaplasmosis.

Materials and Methods

Experimental animals – the age of the examined dogs was between 2 and 7 years, both genders, from different breeds (3 animals were German Shepherd, 6 – Cocker spaniel, 8 – Bulgarian Shepherd Dog, 10 English Bulldog, 3 – Rottweiler, 9 – Bologne and the rest 121 were without breed).

Positive animals for anaplasmosis were from Kardzhali, Haskovo, Harmanli, Dupnitsa, Vidin and Sofia (neighborhood Musagenitsa, Strelbishte) while the positive for heartworm were the regions of Sofia (neighborhood Suha Reka), Radulovtsi village, Novi Iskar, Lom and Silistra.

Methods – the used tests were IDEXX SNAP 4Dx Plus Test and Cani V-4 Test Kit Bionote (antigen test for Dirofilaria immitis and antibody test for Borrelia burgdorferi, Ehrlichia canis or Ehrlichia ewingii, Anaplasma phagocytophilum and Anaplasma platys).

The microfilariae could be identified microscopically through different methods: direct microscopic examination of blood with an anticoagulant; concentrating with colored or uncolored Millipore filter; modified method of Knott (2).

The microfilariae in positive animals were discovered by using of a direct microscopic examination of blood with an anticoagulant (EDTA) with zooming 10x0.25/40x0.65.

From the positive samples for anaplasmosis was prepared colored Romanovski-Giemsa blood smear, which was examined under microscope immersion with zooming 100x1.25 for detection of morules in the platelets and granulocytes.

Results

For the period from 2014 to the beginning of 2016 in the clinic were made 160 tests for vector transmitted diseases. They established twelve positive animals for Dirofilaria immitis, fourteen positive animals for anaplasmosis and ten positives for ehrlichiosis. Five of the examined animals were positive for both Dirofilaria immitis, and anaplasmosis. Lyme disease was not registered.

Dirofilariosis

In 9 of the positive animals for Dirofilaria immitis microscopically were discovered alive microfilariae.

Clinical case 1

Dog named Sandra, 2 years old, weight 35 kg, gender - female, mix-breed, from Radulovtsi village (municipality Slivnitsa). The dog was brought for prophylactic examination and annual immunization. During the clinical examination we determined the internal body temperature – 38.6 °C; the animal occupied unnatural posture – it was holding its elbows away from the thorax and it was presented hyperventilation. The visible mucous membranes were mild cyanotic. During auscultation of lungs we heard abnormal noises (crackling) above the caudal lung lobes.

Clinical case 2

Dog named Toncha, 3 years old, weight 31 kg, gender – female, mix-breed, from Sofia (Suha Reka neighborhood). The dog was brought with a history that for the last 3 weeks even after the slightest exertion it gets tired very fast. In the last week the dog started coughing too. In the clinical examination we established hyperventilation and cyanotic mucous membranes. Auscultation of the heart and lungs showed splitting of the second heart tone and inspiratory breathlessness.

Clinical case 3

Dog named Richy, 5 years old, weight 45 kg, gender – male, breed German shepherd, from Novi Iskar.

The dog was brought for examination, because for 3 days it refuses eating, it drinks a lot of water and it is lethargic.

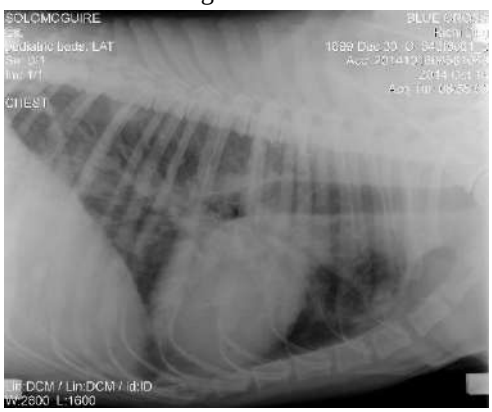

Figure 1: Lateral projection of lung.

Figure 2: Positive for Dirofilaria immitis SNAP 4Dx Plus® test.

The clinical examination showed internal body temperature 40,1° C, expressed cyanosis of the mucous membranes, dry and brittle coat, dehydration, prolonged capillary filling time. The auscultation showed wheezing and breathlessness and the heard tones could not be distinguished clearly. The chest X-ray showed (Fig. 1.) reduced transparency of the lung parenchyma, increased pulmonary vessels, shading in the hilar area and right-sided cardiomegaly. The x-ray signs were typical for Caval syndrome which is typical for the fourth degree of dirofilariosis. The owners refused treatment.

The tests SNAP 4Dx Plus® in these animals were positive for Dirofilaria immitis (Fig. 2). In the first two patients we observed alive microfilariae in the blood smear, made of peripheral blood.

Treatment:

In all the animals positive for Dirofilaria immitis with no evidence of development of Caval syndrome and ascites changes were implemented the following treatment scheme:

Kepromec® (Ivermectin 10 mg/ml) in dosage 0.05 mg/kg p.o., once per week for three consecutive weeks; from the second week – Doxycycline® (Stada) in dosage 10 mg/kg on every 12 hours for the next 4 weeks; after the third week – Melarsomine in dosage 2.5 mg/kg in the area of L3–L5, deep intramuscular three times (with an interval between the first and second treatment 24 hours and the third – after 1 month).

Figure 3: Negative test for dirofilariosis.

As adjunctive therapy in order to prevent anaphylactic reactions and reduction of pulmonary thromboembolism the animals were given Prednisolone (Prednivet®) in dosage: the first week 1mg/kg daily, the second week – 0.5 mg/kg daily; the third week – 0.25 mg/kg daily.

Three months after the last injection with Melarsomine we did control test, which was negative (Fig. 1). and in the blood were not found alive microfilariae.

Anaplasmosis

Fourteen of the all the examined animals were positive for Anaplasma phagocytophilium (Fig. 4). In the smears were found inclusions – morules in the cytoplasm of the granulocytes (Fig. 5), which confirmed the result of the chromatographic immunoassay tests.

Figure 4: Positive test for anaplasmosis.

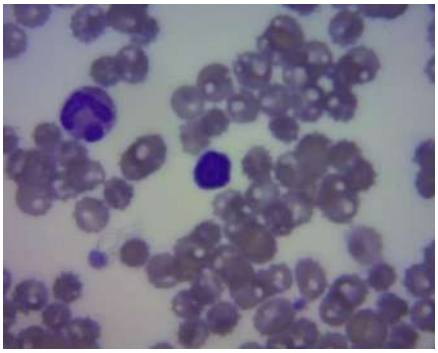

Figure 5: Granulocyte with inclusions in the cytoplasm -morules of anaplasma phagocytophilium.

Clinical case 1

Dog named Jessy – 4 years old, weight 28 kg, gender – female, mix-breed, from Sofia (neighborhood Musagenitsa).

The dog lives outside and came with a history that from three days it refuses eating lacks of desire for playing and prefers to lie down.

During the palpable examination of the abdominal area and joints the animal showed pain. Retropharyngeal lymph nodes were enlarged, but not painful. The activity of the liver enzymes was increased (ASAT 41 U/l, ALAT 66 U/l, AF 171 U/l) and hypoproteinaemia was observed (TP 50 g/l).

Clinical case 2

Dog named Mili – 12 years old, weight 9 kg, gender – female, breed Cocker-spaniel, from Sofia (neighborhood Strelbishte).

The dog is living in apartment. It was brought for examination because it refuses to eat, it vomits and shows apathy. Treatment against ectoparasites was made only in the event that these have been seen by the owners.

Clinical examination showed severe icterus, petechial haemorrhages on the mucous membranes and palpable reaction of pain in the abdominal area. The blood examination showed leuko-

cytosis (27.4 Hx10^9/L) with granulocytosis (23.3x10^9/L), erythrocytopenia (4.8x10^12/L), hemoglobinaemia (106 g/L), thrombocytopenia (81x10^9/L) and increased activity of transaminases (ALAT 132 U/l, ASAT 98 U/l, AF 371 U/l).

Clinical case 3

Dog named Barney, 2 years old, weight 8 kg, gender – male, breed – bolognese. The animal was adopted two days ago from Vidin. The new owners have noticed increased thirst and cough.

Pathological clinical signs included enlarged and non-painful retropharyngeal lymph nodes, increased bronchial breathing and expiratory dyspnea.

Hematological examination showed lymphopenia (0.3x10^9/L) and increased activity of transaminases (ALAT 80 U/L, ASAT 56 U/L, AF 176 U/L).

Treatment:

In the positive animals for anaplasmosis we performed the followed treatment:

Imochem-120® (Imidocarb dipropionate 120 mg/ml) in dosage 5.5 mg/kg, i.m. The second treatment was after 14 days in the same dosage; Additional non-specific therapy included Dexamethasone® 0.2 % (Alfasan) - 0,5 mg/kg, i.m. and Amoxicillin® 20 % (Alfasan) – 7 mg/kg, s.c for a period of 4 days after the treatment with Imochem-120. Hepatoprotection was performed withTransmetil® (Ademetionine 500 mg tabl.) in dosage 10 mg/kg.

One month after treatment the control test was negative (Fig. 6) and no inclusions were found in the granulocytes (Fig. 7).

Figure 6: Negative test for Anaplasma.

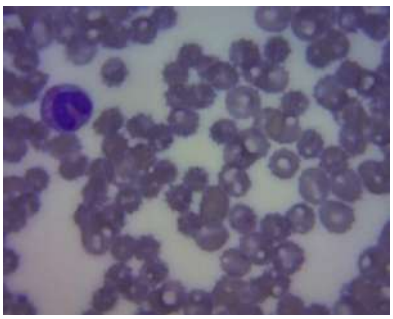

Figure 7: Granulocyte with no pathological inclusions in the cytoplasm.

Discussion

The application of combined scheme for treatment of dirofilariosis with Melarsomine, Doxycycline and Ivermectin is intended to act on both adult and larval forms and reduce the reproduction of the parasite.

Melarsomine is indicated for treatment and stabilization of CHD in 1st, 2nd and 3rd stages of the disease. Melarsomine is an arsenic compound and the exact mechanism of action is unknown. In laboratory and field condition is established 90–99 % efficiency of the preparation with regard to the destruction of adult parasites and L5–larvae in dogs. The product is contraindicated in class 4 of CHD (Caval syndrome) (Donald P., 2012).

It has been found that doxycycline has bacteriostatic act by inhibiting protein synthesis and reducing population of microorganisms of the genus Wolbachia, associated with the dirofilariosis. Administration at the dose of 10 mg/kg, two times

daily for 4 weeks reduces by 90 % this kind of microorganisms, which hinders reproduction of the heart worms (Kramer L. et al., 2005).

Ivermectin at a dose of 50 μg/kg gradually destroys larval forms (L3 and L4) and microfilariae of D. immitis and reduces the duration of life of adults (Donald P., 2012). Using of high doses may increase the risk for the patient and can lead to undesired side effects (Kittleson, M., 2006).

The treatment we used for established by us sick animals with dirofilariosis showed 100 % effectiveness in the described doses and treatment period.

The second therapeutic scheme including Imidocarb (Artemisinin) was used in animals with anaplasmosis.

Imidocarb (Artemisinin) is an antiprotozoal mean used in veterinary medicine primarily for treatment of babesisis. In the last years the high interest of Artemisinin in human medicine is because of the establishment of its powerful anticancer abilities in cell lines and animal models. On the other hand, this product is being imposed as primary antiparasitic agent in intracellular intestinal parasitic diseases and malaria in humans (O'Neill P. et al., 2010).

The mechanism of action of artemisinin is not fully explored but the main hypothesis is related to damaging to the DNA molecules of the target cells. Artemisinin causes impairing of the structural orientation of the DNA molecule and subsequent denaturation, leading to impaired cellular recovery and replication. Side effects are associated with pain and transient cholinergic effects (salivation and vomiting) (Donald P., 2012).

In conclusion our clinical study showed that the application of the examined schemes for treatment of dirofilariosis and anaplasmosis are curative. The usage of the specific drugs (Melarsomine and Imidocarb) leads to the excellent therapeutic results.

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