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CANINE DIROFILARIASIS: A CASE REPORT AND REVIEW OF THE LITERATURE

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ABSTRACT

Dirofilariasis is a disease of clinical importance in dogs. It is to this end that a case of a seven-year-old mongrel with dirofilariasis was presented to examine the unique features and presentation in the canine patient in question. The dog had clinical signs consistent with the disease. Further diagnostic tests were performed to establish the presence and severity of the disease and make an appropriate treatment plan. The Knott's test revealed *Dirofilaria* species in the circulating blood and radiography showed right ventricular hypertrophy with pulmonary arterial enlargement and increased bronchial opacification. The treatment instituted was ivermectin therapy by subcutaneous injection every two weeks for six months, cardiac glycoside and antibiotics for 14 days. The clinical signs resolved after completion of the treatment. A general overview of heartworm infection in dogs was also done to update current knowledge of the disease. Though the risk of significant propagation of *Dirofilaria immitis* is considered low, with the climate change and international pet travel regulations, this emerging zoonosis remains a threat.

Key words: case report; *Dirofilaria immitis*; dog; Nigeria; zoonosis

INTRODUCTION

Etiology and Epidemiology

Canine dirofilariasis, also called heartworm disease, is a non-contagious, parasitic disease caused by a filarial/small thread-like worm, *Dirofilaria immitis* of the family *Onchocercidae* [20]. The parasite is one of the most pathogenic nematodes distributed worldwide, primarily affecting dogs and cats [34]. Mosquitoes (*Aedes, Culex, Anopheles* species) are the vectors which transmit this parasite from host to host [18]. Alongside *D. immitis* is another filarial worm, *D. repens*, and both are considered to have great clinical importance. They are relatively widespread in many parts of the world and have significant zoonotic potential [35].

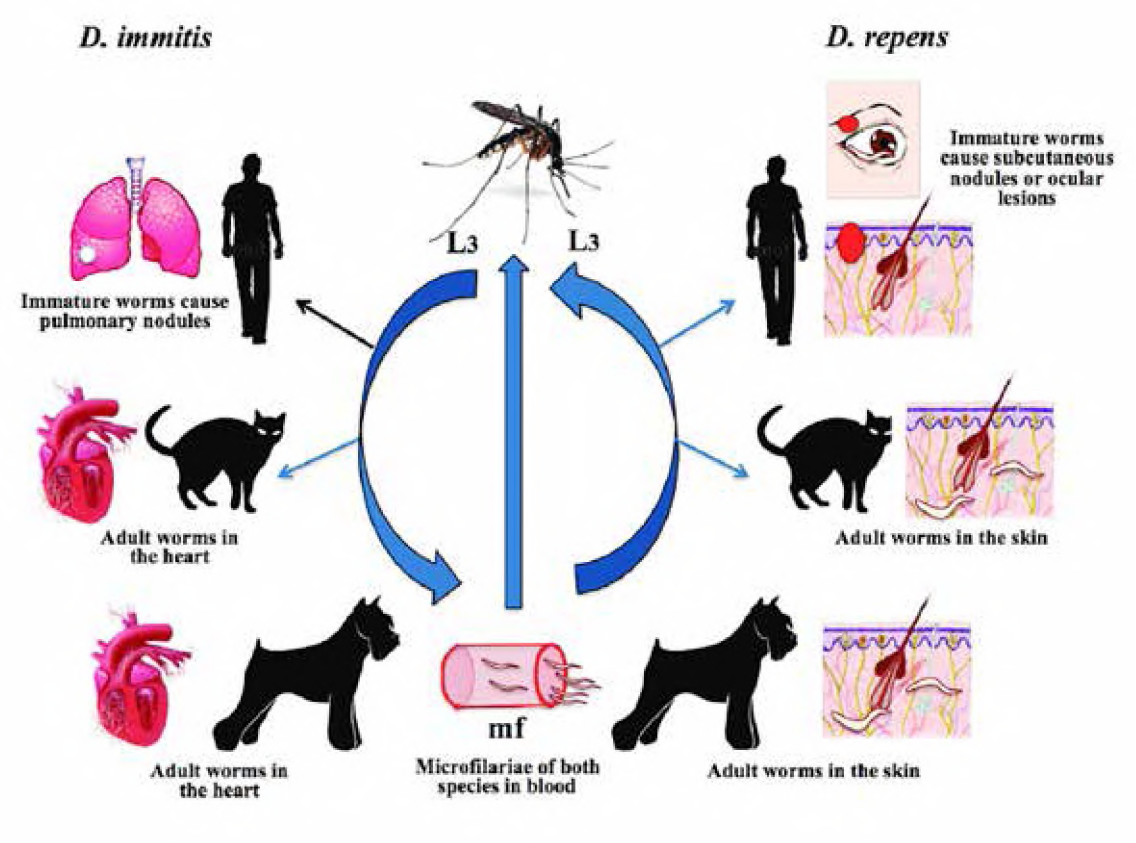

Fig. 1. Biological life cycle for *Dirofilaria immitis* and *Dirofilaria repens* [35]

Dirofilaria immitis establishes itself in the cardiovascular system of the primary host, while *D. repens* invades the subcutaneous tissues of the primary host (Fig. 1) [35].

Pathophysiology

Dogs are considered the definitive host for *D. immitis*, though, more than 30 animal species (e. g. coyotes, foxes, wolves, domestic and wild felids, ferrets) and humans (accidental hosts) may be infected [2, 17]. Adult worms mostly dwell in the right ventricle and pulmonary artery, but occasionally can be found in the epidural space, brain, anterior chamber of the eye, lungs or the arterial system [5].

Microfilariae, larvae in the first larval stage (L1), are produced by the female adult worm and released into the bloodstream of the primary host [31]. Further development of the microfilariae must take place in the vector after taking a blood meal from the host [35]. The development from L1 to L3 can occur in different mosquito species, requiring an average temperature of about 14—18 °C over a period of 30—60 days [8, 16]. The infective larval stage L3, is transmitted through inoculation into the skin of another primary host by the vector and undergoes development from L3 to L4 in the host's subcutaneous tissue in about 10—12 days [35]. Development from L4 to L5 takes place in the muscles of the host 50 to 70 days after injection [16]. Juvenile worms (L5) penetrate the systemic veins and are transported to the pulmonary arteries where they continue to develop into adult worms. In severe cases, worms may also enter the right heart chamber and caudal *vena cava*, but in most cases, they are retained in the pulmonary artery and its branches [14].

The maturation of the female worms in the pulmonary arteries of the primary host cause inflammatory reactions in the pulmonary microvasculature and larger arteries of the hosts [16]. The pulmonary artery expands in diameter, the endothelium and tunica media thicken, and the blood vessels may become obstructed by formation of thrombi causing pulmonary hypertension [35]. The presenting lesions of right ventricular enlargement, pulmonary vasculature dilation and parenchymal lung infiltrates are consistent with heartworm disease [3] (Fig. 2). As the disease progresses, the clinical signs seen will depend on the duration of the infection, the worm burden in the host and the host-parasite interactions [1]. This eventually culminates into a multisystemic disorder affecting the heart, lungs, liver and kidneys [30].

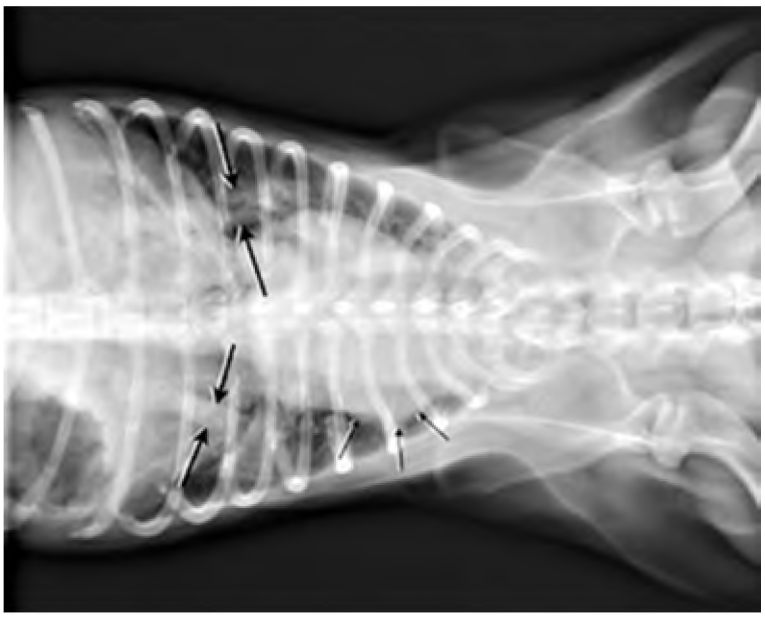

Fig. 2. Ventro-dorsal thoracic radiograph showing blunted, enlarged and tortuous pulmonary arteries (large black arrows) and an enlarged pulmonary trunk (small black arrows) [3]

Prevalence and risk factors

The prevalence depends on many factors, such as the methods performed and preselection of the samples; therefore, different prevalence reports exist within and between countries. The prevalence of *D. immitis* is reported to be 1.0 % in South Australia [10], 5.5 % in Brazil [25], 19.0 % in Spain [19], 20.9 % in South Korea [29] and 30.8 % in South Africa [26]. In Nigeria, reports of the prevalence of dirofilariasis vary from 4.8 % as reported in Northeast Nigeria [11], 3.36 % in Eastern Nigeria [34] and 2.15 % in the Middle belt of Nigeria [22]. This has been proposed to be due to an increase in dog ownership, effects of stray dogs, international pet travel etc.

Factors determining the prevalence of *D. immitis* in an area include: climate, mosquito species/subspecies and the density of the primary host population [16]. Risk factors for infection of the primary host include: animal species (dogs are the natural primary host), sex (male dogs are more vulnerable), habitat (dogs who live outdoors are more exposed to infection), the size of the primary host (large dogs are more likely to become infected than small dogs), and age due to the longer period of exposure to mosquito bites [12].

Clinical signs

The clinical signs of heartworm disease are due to the damage caused to the organs in which they are established e.g. the pulmonary arteries, the right heart chamber, eyes, kidneys, central nervous system and subcutaneous tissue [17]. The signs can be mild, moderate or severe. The mild signs include: cough, chest pain, hemoptysis, low-grade fever, chills and malaise. The moderate signs are: dyspnoea, coughing, weight loss, syncope, and other signs of right-sided heart failure [17]. Caval syndrome can be seen in severely affected dogs, where worms invade the right ventricle and caudal *vena cava*. This interferes with the closure of the tricuspid valve impeding normal blood flow, leading to cardiovascular collapse. Dogs may also present with intravascular haemolysis, hemoglobinuria and acute general malaise [15].

In some cases, there could be occult infections in which the microfilariae in the circulating blood of infected dogs are not seen. This may be due to: single sex worm infection (male or female), the presence of immature females during the prepatent period of development, geriatric infection or ectopic infection [6].

Diagnosis

Direct diagnosis of heartworm infection can be done by microscopic identification of microfilariae in the blood, while indirect infection can be diagnosed using several blood tests to determine the presence of antigens and/or microfilariae in the host. The presence of worms in the heart or subcutaneous tissues during surgery or post-

mortem is definitive [27]. Laboratory examinations using parasitological (Buffy coat, wet mount, modified Knott's test) and serological (ELISA) techniques may aid the diagnosis. Molecular testing, such as the polymerase chain reaction (PCR) and tissue histochemistry, can help differentiate microfilaria species [16]. Other diagnostic procedures such as general blood tests, radiography, echocardiography and electrocardiography should be performed to determine the degree of severity and serve as a guide in the treatment regimen.

Prophylaxis, treatment and control

Wolbachia pipientis, an intracellular bacterium, lives in symbiosis with *D. immitis*. Studies have shown that the inflammatory reaction occurring during heartworm infection is partly due to *W. pipientis* [32]. Therefore, the use of an anthelminthic agent in combination with an antibacterial agent is strongly recommended [24, 33]. Melarsomine dihydrochloride, an organic arsenical, is the most used anthelminthic agent. It is administered at a dosage of 2.5 mg.kg^{-1}, deep intramuscular injection in the lumbar muscles, twice within a 24-hour interval. Ivermectin, a macrocyclic lactone, at 200 µg.kg^{-1} can be given as a single dose subcutaneously, four to six weeks after treatment with melarsomine dihydrochloride [8]. Doxycycline, an antibacterial agent, will reduce the burden of *W. pipientis* in all stages of *D. immitis* infections.

Prophylactic treatment of all dogs living in areas exposed to infections is recommended to reduce the number of microfilariae in the bloodstream, and thereby curtail transmission. The treatment consists of a monthly dose of orally administered milbemycin or topically applied ivermectin.

CASE PRESENTATION

On the 17th of September, 2018, a seven-year-old, female mongrel was presented to the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta, Nigeria, with complaints of frequent episodes of coughing for about two years. The coughing became persistent and severe about two weeks before presentation. The pet had been placed on multivitamins and a teaspoon of cod-liver oil given four times daily in food orally before presentation. Different antibiotics such as oxytetracycline, penicillin-streptomycin and tylosin had been prescribed for the pet at different times by different veterinarians.

On clinical examination, the pet weighed 17 kg, rectal temperature was 40.2 °C, and the heart rate was 81 beats per min. The ocular mucous membranes were pinkish and there was no enlargement of the superficial lymph nodes. Heart auscultation revealed arrhythmia, although the femoral pulse was strong and regular. Capillary refill time was one second and the jugular vein appeared normal without visible pulsations. The dog was panting and coughing to some extent in the examination room and auscultation of the lungs revealed severe crackles on both sides of the chest.

A tentative diagnosis of bronchopneumonia and dirofilariasis was made. The differential diagnoses included: congestive heart failure, endocarditis and pericarditis. The blood was obtained for haematology and parasitological analysis. The pet was also scheduled for chest x-ray (lateral and ventro-dorsal views).

The haematology was within normal limits (Packed Cell Volume, PCV—50 %; total white blood cell count, WBC—$10.8 \times 10^{9}.l^{-1}$), no parasite was found in the blood film using the wet mount and thin smear techniques. On day two of the presentation, Knott's test was carried out on the blood sample and *Dirofilaria* species was found microscopically (Fig. 3).

The radiography results showed right ventricular hypertrophy with pulmonary arterial enlargement, and increased bronchial opacification (Fig. 4). Based on the medical history, clinical signs and the presence of the worms, a confirmatory diagnosis of canine dirofilariasis was made. The dog was classified as moderately affected.

Management and outcome

Treatment included 300 µg.kg^{-1} ivermectin (Ivomec®, Merial) administered subcutaneously every two weeks. In addition, 2.5 mg (1/2 tab.) enalapril (Lotrial®, ATOZ Pharmaceuticals, India) orally once daily and 0.125 mg (1/2 tab.) digoxin orally once daily was administered for 14 days. After treatment daily, the dog was admitted to the veterinary clinic for observation for six hours. The dog was released from the clinic in the evenings, for observation by the owners. Exercise was limited the first few weeks after each treatment. No side effects were observed following the treatments.

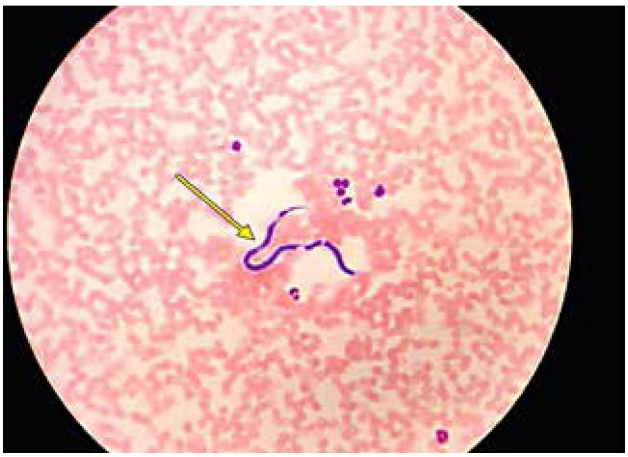

Fig. 3. *Dirofilaria immitis* (Arrow). Magnification ×100 of the microscope with a phone (Infinix) camera from top. The anterior end of *Dirofilaria immitis* tapers anteriorly as seen with the Knott's method which is the standard for diagnosis of canine dirofilariasis [7]

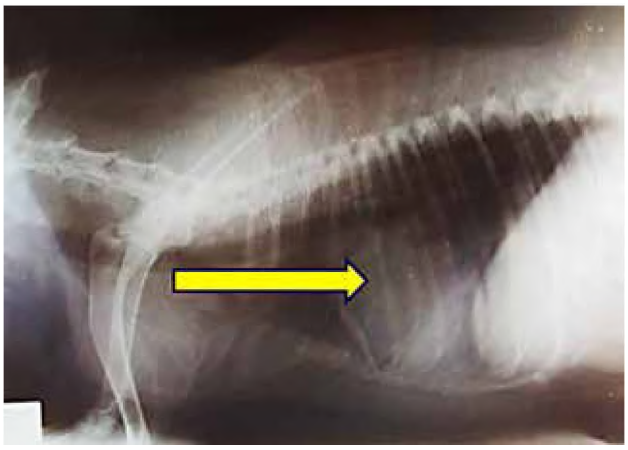

Fig. 4. The lateral thoracic radiographic view of the animal showing right ventricular hypertrophy with pulmonary arterial enlargement and increased bronchial opacification consistent with canine dirofilariasis

By day 14, the dog's clinical signs had improved and there was no microfilaria detection in the blood after the first shot of ivermectin. The dog continued to have a mild cough at this stage, and was treated with doxycycline (Ronaxan vet®, Merial) 10 mg.kg^{-1} once daily for 14 days which was initiated to take care of the potential concurrent infection with *W. pipientis*. Ivermectin therapy at 300 µg.kg^{-1} was continued every two weeks for six months.

Three months later, a serological antigen test was carried out (immunoassay, Laboklin Laboratories, Germany) and the test was negative. Six months later, the dog was presented to the clinic in very good physical condition and had stopped coughing. The breathing pattern and respiratory rate were normal.

DISCUSSION

This case of canine dirofilariasis in a seven-year-old patient was diagnosed based on the history, clinical signs observed, the laboratory investigations (PCV, CBC, Knott's test) and radiographic findings. This agrees with V a t n e [35] who reported canine dirofilariasis in a three-year-old dog. With a prepatent period of about six months, where female *D. immitis* worms grow between 13.5 to 30.5 cm long and can start producing microfilariae, it is unlikely to see clinical signs in dogs younger than one-year-old [8].

The long duration of coughing episodes in the bitch coupled with the previous administration of tylosin without remission aided in the diagnosis of this case. An adult worm can survive for up to seven years in the primary host, while microfilariae have a lifespan of up to two years, hence the long duration [27]. Clinical signs seen are consistent with other cases of moderate canine dirofilariasis reported [4, 13, 23]. Eosinophilia is most often seen in dirofilariasis which is caused by the body's cellular response to the circulating microfilaria in the blood vessels.

The modified Knott's method [28] was used for the diagnosis of the blood parasite in this case, a method that is more sensitive than a thin smear in diagnosing microfilariae and it helps in concentrating microfilariae for easy identification. The parasitological technique is a type of concentration test used in screening parasites in blood of low parasitaemia and this technique is used to distinguish between *Dirofilaria* and *Dipetalonema* species. A stationary rather than a migratory pattern of movement is indicative of *Dirofilaria* species when viewed under the microscope. Another diagnostic technique used to aid in diagnosing this case was radiography. The lateral view of the thoracic region of the pet showed enlargement of the right chamber of the heart and pulmonary artery, as previously reported by B a r r et al. [3].

Ivermectin, a macrocyclic lactone, was administered as an endectocide, subcutaneously once every two weeks. It works by the activation of glutamate-gated chloride channels, preventing the secretion of immunomodulatory proteins by the parasite, resulting in increased vulnerability of the worm to attack by the host's immune system [36]. According to N e l s o n et al. [21], the use of ivermectin can be used to treat the adult stages of the worm. Enalapril

and digoxin were dispensed as a supportive treatment in this case to reduce and alleviate the cardiopulmonary signs in the pet. Enalapril, an angiotensin converting enzyme inhibitor, is indicated for the treatment of congestive heart failure and hypertension. This drug, whose use in dogs in well tolerated, acts by preventing the formation of angiotensin-11 (a potent vasoconstrictor) by competing with angiotensin-1 for the enzyme angiotensin-converting enzyme. [9]. Digoxin, a cardio-tonic glycoside, also indicated for use in the treatment of congestive heart failure in dogs was administered to the dog. The drug acts by increasing myocardial contractility with increased cardiac output.

CONCLUSIONS

A case of canine dirofilariasis based on the history, clinical findings, laboratory investigations and radiography were presented. Veterinarians, physicians, pathologists and parasitologists should have an increased awareness of this entity. Various epidemiological and diagnostic means for the identification and molecular characterization of the species, and natural hosts can help to establish low prevalence rates of this emerging zoonosis and devise control measures.

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