



Diagnosis of Microfilaria Infection in dog with Geimsa stain

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Introduction

Dirofilaria immitis, also known as dog heartworm, is one of the most important filaroid nematodes responsible for canine dirofilariasis. The heartworm parasite is spread by mosquitoes from the genera *Culex*, *Aedes*, and *Anopheles*. Adult female *D. immitis* lays microfilaria, which are picked up by mosquito vectors and develop into the infective third larval stage. When a potential vector bites dogs or other hosts during a subsequent blood meal, transmission occurs. It takes approximately 6-7 months to reach the adult stage. The presence of an adult worm causes the pathophysiological response to heartworm infection. The most common clinical symptoms of dirofilariosis are a persistent cough, difficulty breathing, and poor nutrition. In terms of demonstrating and identifying microfilaria in tested blood samples, laboratory diagnosis (Giemsa stain technique) of dirofilariosis in live animals is always at the forefront. Although radiography and cardiography aid in the diagnosis of *D. immitis*, serology and molecular tests are required for a definitive and reliable diagnosis of heartworm disease.

Case History

In this report blood sample from a 6-year-old Labrador dog was collected which was reported by owner from Barapani, Umroi road to the ICAR Research Complex Umiam, Meghalaya, and had a previous history of anorexia, weakness, coughing, and dyspnea. During the physical examination, the dog had shown debility, pale mucous membranes, a high rise in body temperature (39 degrees Celsius), laboured breathing, and weakness but had normal respiratory and heart rates. With the help of a disposable syringe, a blood sample (2 ml) from the cephalic vein of the infected dog was collected in an anticoagulant-containing (EDTA) vial. The blood sample was examined with the Giemsa stain technique. In the Giemsa stain technique, a thin blood smear is made on a slide and stained with Giemsa stain, and the slide is kept for air



drying. The presence of microfilaria was confirmed under oil immersion (100X) and which was found an elongated snake-like structure in the erythrocytes shown in the figure below.



Figure: Shown the microfilaria worm under the Geimsa stain technique.

Treatment

Effective treatment of doxycycline at rate of 10 mg/kg orally twice daily for 30 days and combined treatment with ivermectin 6 mg/kg orally for weekly interval recovered eventually in 30 days after beginning of the treatment.

Result and Discussion

The used of effective method of blood smear with Geimsa stain diagnosed the positive result of microfilaria infection.

In the present case report study, revealed that the vector borne disease such as microfilaris are endemic in the sub-tropical region including north east India like Meghalaya, Mizoram and Assam. These states are vulnerable to climate change characterized by increased rainfall and environmental temperature resulting likelihood increase of vector borne diseases including human malaria. *Microfilaria immitis* (Nematode: Filariidae) causes heartworm disease, which is widespread throughout tropical and temperate regions of the world including Europe stated by **Mario Santoro et al. (2019)**. Transmitted from host-to-host via mosquito bites and the prevalence of the disease is influenced by climate and topography, but most importantly, by the presence of mosquito vectors, **Nguyen et al. (2016)**. The owner from Barapani, Umroi road was reported a Labrador dog to the ICAR Research Complex Umiam, Meghalaya and the owner also informed the bites of mosquitoes and the dog was shown the symptoms of microfilariasis and in this report the result found was also similar with **Sonjoy Kumar Borthakur et al (2014)**. He was also found the main clinical symptoms in dirofilariasis include persistent cough, difficult breathing, and poor exercise tolerance followed by ascites, anorexia, and weight loss. In this report, the history of mosquito's bite which was similar with finding by **A.L. Vieira et al, 2014**. Where they had reported the different species of culicid mosquitoes belonging to the genera *Culex*, *Aedes*, and *Anopheles*, among



others, have been implicated in the transmission of this parasite, allowing for its intermediate stage to complete its life cycle. In the present case report micro filariasis was diagnosed with the helped of Giemsa staining technique as shown in the figure, microfilaria under the oil emersion (100X). This was similar with Ibeh Nnanna Isaiah et al. (2021). Were identified *D. Immitis* with the helped of Giemsa-stained blood smears technique. At presence case report the dog was advised to treat with doxycycline at rate of 10 mg/kg orally twice daily for 30 days. Blood samples were collected every 30 days during 6 months of treatment and combined treatment of doxycycline with ivermectin 6 mg/kg orally for weekly was given. This treatment result was also similar with Amarjit et al. (2017), where the combine treatment of *Microfilaria* infection in dog with ivermectic injection of at the rate of 0.3mg/kg body weight subcutaneously weekly for four occasions with doxycycline the rate of 10mg/body weight orally for one month was effective.

Summary

The used of effective method of blood smear with Geimsa stain diagnosed the positive result of microfilaria infection.

Conclusion

Dirofilaria immitis, a parasite of the cardiovascular system of carnivores found all over the world, has been found in dogs, cats, and hu mans. Mosquitoes are among the arthropod vectors that transmit *Dirofilaria immitis*. In the laboratory, the diagnosis of *Dirofilaria immitis* can be done with different techniques, like the Giemsa stain technique, the Modified Knott's Technique, ELISA, PCR etc. To prevent the infection from *Dirofilaria immitis*, we should take precautions, like the use of mosquito net for the dog, the use of some mosquito repellent spray, and most importantly it is necessary to keep clean and hygiene of surrounding household.

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