CLINICO-THERAPEUTIC STUDIES ON CANINE HEARTWORM DISEASE

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Canine heartworm disease (dirofilarosis) is caused by *Dirofilaria immitis*. Most affected dogs were asymptomatic but the most common clinical features of the disease were progressive weight loss, exercise intolerance, laboured breathing and coughing. Haemogram of the affected dogs revealed marked leukocytosis, eosinophilia and thrombocytopenia. Serum biochemical investigations showed significant increase of alkaline phosphatase, ALT and serum creatinine whereas serum glucose levels in the affected dogs were found significantly decreased. Results also indicated that combined administration of ivermectin @ 0.3 mg/Kg BW orally at two weeks interval for 6 weeks, doxycycline @ 10 mg/Kg BW orally once daily given for 4 weeks and prednisolone @ 0.5 mg/Kg BW orally once daily for first 10 days followed by on alternate days for next 10 days was found effective. Affected dogs showed remarkable reduction in circulating microfilaria along with good improvement of clinical signs.

Keywords: Canine heart worm disease, *Dirofilariaimmitis*, Clinical features, Haemato-biochemical alterations, Combined therapy.

Canine heartworm disease (CHWD) (dirofilariasis) is caused by *Dirofilaria immitis*. Which is also known as heartworm. It is one of the important parasitic threats for dogs around the world. Canine heartworm disease adversely affects the health and wellbeing of dogs in most of the countries with temperate, semitropical or tropical climates such as North America, Europe, Australia and Asia (Simon *et al*., 2012). Disease is also prevalent in India (Rathore and Sharma, 2022). CHWD is a vector borne disease. *Dirofilaria immitis* has zoonotic potential and thus could be considered as a public health issue (Fontes-Sousa *et al*., 2019).

Mosquitoes particularly *Aedes*, *Anopheles*, and *Culex*are the most commonly act as vectors. Mosquito acquire microfilariae during feeding on an infected animal. Infective larvae (L3) molt into the fourth stage (L4) within 3–12 days in dogs. This L4 undergo their final molt at 50 to 70 days into young adults, arriving in the heart and pulmonary arteries. These worms rapidly grow to adult worms in the pulmonary vasculature. As the parasites grow, it occupies larger pulmonary arteries and occasionally moves into the right ventricle and even the atrium when the worm burden is quite high. Gravid females produce microfilariae at 6-9 months after infection.

The role of the endo-symbiotic bacteria *Wolbachia spp.*, which lives intracellularly within the filarid parasite, is still to be determined. However, *Wolbachia spp.*has been implicated in the pathogenesis of canine heartworm disease, possibly through endotoxin production. The most important aspect of bacteria *Wolbachia spp.* is its symbiotic relation with *D.immitis*. This bacterium is required for maturation, reproduction and infectivity of the heartworm (Ferri *et al*., 2011; Taylor *et al*., 2013). With elimination of *Wolbachia*, the heartworm first become sterile and then gradually dies. This can be accomplished with doxycycline therapy, which has become an important part of the armamentarium against heartworms (Turner *et al*., 2020). Treatment of canine heartworm disease in dogs requires a prolonged regimen of treatment along with exercise restriction (Carreton *et al*., 2019).

The objective of the present investigation was to find out the clinical features, haemato-biochemical alterations and evaluation of combined ivermectin, doxycycline and prednisolone therapy.

Materials and Methods

Dogs presented in clinics and visited at

households were examined for canine heart worm disease. The inclusion criteria for the study were all dogs visited and being subjected to a clinical examination, regardless of original history. The dogs' breed, sex and age were recorded. Dogs suffering from canine heart worm disease revealed a wide variety of clinical manifestations. Affected dogs showed the symptoms of dullness, depression, decreased appetite, unthriftiness, reluctance to exercise, mild persistent cough, dyspnea, haemoptysis, ascites, cyanosis, progressive weight loss and cardiac murmurs on auscultation. Conjunctiva was pink in color. Thorough clinical examination of the dogs revealed significant increase in rectal temperature, respiration rate and heart rate which accompanied with arrhythmia. The mean temperature, heart rate and respiration rate were 103.2 ±1.30^{0}F, 130.6±3.60 per minute and 42.5±2.00 per minute, respectively.

The dogs under present investigation were subjected to detailed clinical examination and the clinical signs observed were recorded. Whole blood was collected from each animal in to a vacutainer containing Buff. Na. citrate (9NC) for haematological and parasitological examination. Blood smears were examined for presence of microfilaria of *D. immitis*. Simultaneously, blood was also collected in the serum tubes for separation of serum. Haematological parameters like haemoglobin (g/dl), PCV (%), TLC (10 thousand/mm^3), TEC (10^6/mm^3), differential leucocyte count and platelets count were estimated. The biochemical parameters viz. ALP, ALT, AST, BUN, serum creatinine, total serum protein (TP), serum glucose and serum albumin were estimated using Auto-Biochemistry Analyser-IdexxVetTest.

Mean + SE values of haematological parameters in healthy control dogs and canine heartworm disease affected dogs is presented in Table-1.

The mean±SE value of haemoglobin (g/dl) in dogs affected with canine heartworm disease was significantly ($p<0.05$) decreased (10.60±0.1) as compared to healthy control group (14.8+0.4). The mean value of total leukocyte count (thousand per mm^3) was significantly ($p<0.05$) higher in canine heartworm disease affected dogs (14.05±0.20) as compared to healthy control group (11.50+0.22). The mean value of total erythrocyte count (million/mm^3) was significantly ($p<0.05$) decreased in canine heartworm disease affected dogs (6.20±0.04) as compared to healthy control group (6.82+0.12). Non-significant difference was recorded in mean value of packed cell volume of affected dogs as compared to healthy dogs (Table-1).

The differential leukocyte count (%) in canine heartworm disease showed significant ($p<0.05$) eosinophilia (6.42+0.70 and 2.76±0.20), neutrophilia (72.30±0.56 and 68.4±0.69) and lymphopenia (17.82±0.80 and 23.43+0.62) as compared to healthy control group. A non-significant difference in mean values of monocyte was recorded in canine heart worm disease affected dogs (1.92±0.16) as compared to healthy control dogs (2.26±0.30). Marked thrombocytopenia was observed in canine heartworm disease affected dogs (288±55.28 x10^9/L) as compared to healthy control dogs (188.75±38.44 x10^9/L)(Table-1).

Table-1: MEAN + SE VALUES OF HAEMATOLOGICAL PARAMETERS IN HEALTHY DOGS AND CANINE HEARTWORM DISEASE AFFECTED DOGS

Haematological Parameters	**Healthy Group (n=6)**	**Canine heartworm disease affected dogs (n=6)**
Hb (g/dl)	14.8+0.4	10.60±0.1
PCV (%)	38.22+0.60	36.82±0.62
TEC (million/mm^3)	6.82+0.12	6.20±0.04
TLC (thousand per mm^3)	11.50+0.22	14.05±0.20
Platelet (x10^9/L)	188.75±38.44	288±55.28
Differential Leucocytes counts		

Neutrophils (%)	68.4+0.69	72.30±0.56
Lymphocytes (%)	23.43+0.62	17.82±0.80
Monocytes (%)	2.26+0.30	1.92±0.16
Eosinophils (%)	2.76 ±0.20	6.42+0.70

Mean + SE values of serum biochemical parameters in healthy control dogs and canine heartworm disease affected dogs is presented in Table-2. Serum biochemical values in apparently healthy dogs recorded were within the normal range.

The mean ± SE value of alkaline phosphatase (ALP) (U/L), alanine transaminase (ALT) (U/L) and serum creatinine (mg/dl) in dogs affected with canine heartworm disease were significantly ($p<0.05$) increased (189.66+5.45, 79.40+1.45 and 1.89±0.04) as compared to healthy control group (89.40+4.45, 38.48+6.80 and 0.88+0.22), while statistically significant decrease ($p<0.05$) in the mean value of glucose (mg/dl) was observed in dogs affected with canine heartworm disease (86.62±1.37) as compared to healthy control group (98.6+2.92).

Non-significant difference was recorded in mean value of aspartate transaminase (AST), blood urea nitrogen (BUN), total Protein (TP) and serum albumin canine heartworm disease affected dogs as compared to healthy group.

Table-2: MEAN + SE VALUES OF SERUM BIOCHEMICAL PARAMETERS IN APPARENTLY HEALTHY AND CANINE HEARTWORM DISEASE AFFECTED DOGS

Serum Biochemical Parameters	Healthy Group (n=6)	Canine heartworm disease affected dogs (n=6)
ALP (U/L)	89.40+4.45	189.66+5.45
ALT (U/L)	38.48+6.80	79.40+1.45
AST (U/L)	22.64+2.68	27.60+0.64
BUN (mg/dl)	12.64+2.18	13.12 + 0.66
Creatinine (mg/dl)	0.88+0.22	1.89±0.04
Glucose (mg/dl)	98.6+2.92 mg/dl	86.62±1.37
Total Protein (g/dl)	6.22+0.24	6.52±0.06
Albumin (g/dl)	2.8+0.08	2.6±0.04

Treatment

Treatment regimen included combined administration of ivermectin @ 0.3 mg/Kg BW orally at 2 weeks interval for 6 weeks, doxycycline @ 10 mg/Kg BW orally once daily for 4 weeks and prednisolone @ 0.5 mg/Kg BW orally once daily for first 10 days followed by alternate days for next 10 days was evaluated. Amelioration of clinical signs and worm load was recorded after every two weeks.

The dogs were treated with Injection Meloxicam @ 0.5 mg/Kg BW intramuscular in case of fever. Tab. Doxycycline @ 10 mg/Kg B.Wt. orally once daily was given for 4 weeks along with Tab. Prednisolone @ 0.5 mg/Kg B.Wt. orally once daily for first 10 days followed by Prednisolone @ 0.5 mg/Kg B.Wt. orally on alternate days for next 10 days. Tab. Ivermectin was given @ 0.3 mg/Kg B.Wt. orally at two weeks interval for 6 weeks. Owners were advised to avoid the dog exercise. The dogs showed remarkable reduction in circulating microfilaria along with good improvement in clinical signs after 2 weeks and complete recovery in all cases after six weeks. The statistical analysis of the data was done using statistical methods described by Snedecor and Cochran.

Results and Discussion

Dogs suffering from canine heart worm disease revealed a wide variety of clinical manifestations. Similar findings were reported by Ames and Atkins (2020) and Rathore and Sharma (2022). Canine heartworm disease is initially a vascular disease that can progress to impaired blood flow, eventually affecting the vascular and pulmonary system and also in severe cases the right heart chambers. Damage to the pulmonary endothelium and vascular

occlusion from worms' death will reduce cardiac output. The resulting pulmonary hypertension may lead to compensatory right-sided cardiac enlargement and progress to right sided cardiac failure. As less common sequelae,large numbers of worms in the pulmonary arteries cause sudden obstruction of blood flow through the lungs. This reduced blood flow enables the worms to migrate and become aberrantly located in the right atrium, ventricle and often in the vena cava. This type of blockage is called as caval syndrome and is a life-threatening form of cardiac failure (Ames and Atkins, 2020).

The frequency and severity of clinical signs may be correlated with the lung pathology. Worm death and thromboemboli usually precipitate the clinical signs. Based on the severity of clinical signs, infected dogs are classified into stage I to stageIV. In Stage I, minimal clinical signs are shown by the patient which are not evident to owner. In stage II, mild to moderate signs are seen, most often coughing. In Stage III, animal shows severe signs including dyspnoea, weight loss, exercise intolerance and even cardiac failure. Stage IV include dogs with caval syndrome, in which retrograde migration of worms into the right ventricle, atrium and caudal and cranial vena cava precipitates tricuspid valve insufficiency, Resulting to low cardiac output, haemolysis with pigmenturia, anaemia, and hepato-renal dysfunction leading to death.

The findings of present Haematology and Serum Biochemistry investigation were in agreement with that of Rathore and Sharma (2022). The decreased hematological indices (haemoglobin and total erythrocyte count) recorded in the present observations might be due to the prolonged reduced appetite and/or blood loss from inflammatory responses of the body due to these infections.

The macrocyclic lactones (avermectins) are active against parasites. These are the fermentation products of *Streptomyces avermitilis* as also recorded by Campbell, 2012. Ivermectin showed a broad spectrum activity against parasitic nematodes with both oral and parenteral administration. In addition, it has activity against different arthropods like fleas, lice, mites and some tick species as also reported by Martin *et al*., 2020. Ivermectinis effective against L3 and L4 stages of microfilariae while it is not effective against adult worms but it may reduce their fertility as also mentioned by Martin *et al*., 2020.

Doxycycline mediated elimination of *Wolbachia* spp. in canine heartworm infections demonstrated that bacteria *Wolbachia* are required for filarial larval development, embryogenesis and viability, simillarily repoerted by Turner *et al*., 2020. Further,long-term administration of doxycycline in dogs may be associated with low tolerance and severe gastro-intestinal side effects as also narated by Savadelis *et al*., 2018). For effective treatment, a combination of doxycycline with ivermectin showed a superior microfilaricidal and adulticidal effectas compared to the drugs given alone. Combined ivermectin doxycycline therapy seems to reducethe course of treatment by eliminating *Wolbachia* as also advocated by (Carreton *et al*., 2019).

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