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Therapeutic Efficacy of Doramectin and Levamisole in *Dirofilaria immitis* İnfected Dogs and Changes of Some Blood Parameters

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Dirofilaria immitis ile Enfekte Köpeklerde Doramectin ve Levamizol'un Terapötik Etkisi ve Bazı Kan Parametrelerindeki Değişiklikler

Özet: Bu çalışmada, *Dirofilaria immitis* ile doğal enfekte köpeklerde doramectin ve levamizol'un terapötik etkisi ve bazı kan parametrelerinin sağaltım öncesi-sonrası durumu araştırıldı. Modifiye Knott tekniği kullanılarak incelenen 15 köpekten 11'nin *Dirofilaria immitis* ile enfekte olduğu belirlendi. 11 mikrofilaremik köpek, 2 sağaltım grubuna ayrıldı.Enfekte köpeklerden 5'ine bir hafta ara ile 2 kez 200 µg/kg dozda doramectin subcutan, diğer 6 köpeğe günlük 10 mg/kg dozda 14 gün süreyle levamizol oral olarak uygulandı. Mikrofilerler, sağaltım öncesi ve sağaltım başlangıcından sonraki 3, 7, 11, 14, 21 ve 28. günde sayıldı. Hematokrit (PCV), Hemoglobin (Hb), total (WBC) ve differensiyal lökosit sayıları, serum CK, ALT, AST, LDH ve GGT aktiviteleri ve serum total protein (TP), Na, Cl ve K konsantrasyonları, sağaltım öncesi ve ilk uygulamadan 7, 14 ve 28 gün sonra belirlendi. Sağaltım öncesi, PCV, Hb, ve CK değerlerinde bir artış, lenfosit sayısında bir azalma, diğer parametreler ise genellikle normal sınırlar içinde veya yakınında bulundu. Mikrofiler sayı; saat 18.00 de alınan kan örneklerinde, 9.00 da alınanlardan daha fazla saptandı. Doramectin uygulanan köpeklerde, mikrofiler sayıda azalma, sağaltımdan sonraki ilk 2 haftalık sürede %86.9'dan %93.5'e kadar değişti. Uygulamanın 14. gününden itibaren doramectin'in mikrofilarisidal etkisi azalmaya başladı ve 28. günde % 80.4 olarak belirlendi. Doramectin uygulamasından sonra, PCV, Hb, eozinofil, CK, TP, ve Cl değerlerinde geçici bir azalma, K konsantrasyonunda geçici bir artış saptandı. Nötrofil sayısında 7., lenfosit sayısında 14. günde önemli artış bulundu. Levamizol uygulanan köpeklerde, mikrofiler sayısındaki azalma, sağaltımden sonraki ilk 2 haftalık sürede %87.6-%95.1 bulundu. Uygulamanın 14. gününden itibaren levamizol'un mikrofilarisidal etkisi azalmaya başladı ve 28. günde % 83.9 olarak belirlendi. Levamizol uygulamasından sonra, PCV, Hb, eozinofil, CK, TP, ve Cl değerlerinde geçici bir azalma, ALT, Na ve K değerlerinde geçici bir artış saptandı. Nötrofil sayısında 7., lenfosit sayısında 14. günde önemli artış bulundu. Hafif ve geçici yan etkiler, doramectin uygulanan 1, levamizol verilen 2 köpekte ilk ve ikinci uygulamadan sonra görüldü. Uygulama öncesi tesbit edilen belirgin klinik bulguların uygulamadan sonraki günlerde ortadan kalkmaması, mikrofiler sayısının sağaltımdan sonraki 14. günden itibaren gittikçe artması ve her sağaltım grubundan bir köpeğin otopsisinde canlı, olgun *Dirofilaria immitis*'lerin bulunması, doramectin ve levamizol'un, parazitin olgunlarına karşı etkisinin hiç olmadığını veya yetersiz (levamizol) olduğunu gösterdi.

Sonuç olarak, bu çalışma Van'da köpeklerde *Dirofilaria immitis* enfeksiyonunun yaygın olabileceğini, doramectin'in de yüksek mikrofilarisidal etkisinin olduğunu ve enfeksiyon ve sağaltımın bazı hematolojik ve biokimyasal parametrelerde değişiklikler yaptığını gösterdi. Son yıllarda artan köpek populasyonu ve hareketleri nedeniyle, köpeklerde dirofilariasis, kardiopulmonal hastalıkların ayırıcı tanısında daha çok dikkate alınmalıdır. Başarılı sağaltım ve bu yolla enfeksiyonun yayılmasının önlenmesi için etkili bir adultisidal ilacın piyasada bulunması gereklidir.

Anahtar Kelimeler: Köpek, *Dirofilaria immitis* enfeksiyonu, Doramectin, Levamizol, Kan parametreleri.

Summary: In this study, therapeutic efficacy of doramectin and levamisole in dogs naturally infected with *Dirofilaria immitis* and condition of some blood parameters before and after treatment were investigated. Eleven of the 15 military dogs examined by using the modified Knott's technique were found to be infected with *Dirofilaria immitis*. Eleven microfilaraemic dogs were assigned to two treatment groups. Five dogs were given 200 µg/kg of subcutan dose of doramectin twice one week interval; the other 6 dogs were treated with levamisole orally at dosage of 10 mg/kg as a daily basis for 14 days. Circulating microfilariae were counted before treatment and at 3, 7, 11, 14, 21 and 28 days after starting treatment. PCV, Hb, total and differential leukocyte counts, serum CK, ALT, AST, LDH and GGT activities and serum total protein (TP), Na, Cl, and K concentrations were determined pretreatment and 7, 14 and 28 days after the first drug administrations. Before treatment, there were an increase in PCV, Hb, and CK values and a decrease in lymphocyte count. The other haemotologic and serum biochemical parameters were generally within or near to normal ranges. The microfilaria count in blood samples collected at 18 p.m. was higher than the count at 9 a.m. In the doramectin-treated dogs, reduction in microfilaria count ranged from 86.9 % to 93.5 % during the first 14 days after treatment. From the 14th day of treatment, the microfilaricidal efficacy of doramectin tended to decrease gradually and was found as 80.4 % at posttreatment day 28. There were transient decreases in PCV, Hb, eosinophil, CK, TP, and Cl values and a transient increase in K level after treatment. Neutrophil and lymphocyte counts increased significantly at posttreatment days 7 and 14, respectively. In the levamisole-treated dogs, reduction in microfilaria count was found between 87.6 % and 95.1% during the first 2 weeks after treatment. From the 14th day of treatment, the microfilaricidal efficacy of levamisole tended to decrease gradually and was noted as 83.9 % at posttreatment day 28. PCV, Hb, eosinophil, CK, TP, and Cl levels decreased; ALT, Na and K values increased, transiently after levamisole administrations. Neutrophil and lymphocyte counts increased significantly at posttreatment days 7 and 14, respectively. Mild and transient adverse reactions were observed after the first and second drug administrations in 1 dog treated with doramectin and in 2 dogs treated with levamisole. Because the main clinical signs noted by physical examination before treatment did not disappear posttreatment days, the microfilaria count increased gradually from day 14 after treatment, and live adult heartworms were present at necropsy in one dog from each treatment group, both doramectin and levamisole had insufficient (levamisole) or no adulticidal efficacy.

Based on above results, this study revealed that canine heartworm infection in Van may be widespread, also doramectin had a highly microfilaricidal efficacy, and the infection and treatments induced changes in some hematologic and serum biochemical variables. Owing to increase dog population and movement of dogs in the last years, canine heartworm infection requires more consideration in differential diagnosis of cardiopulmonary diseases. It is necessary to put an effective adulticidal drug on the market for successful treatment and by this means prevention of spreading infection.

Key Words: Dog, *Dirofilaria immitis* infection, Doramectin, Levamisole, Blood parameters.

Introduction

Dirofilaria immitis is the only filarial nematode of the dog that produces clinically apparent disease. Canine heartworm disease caused by *D. immitis* is endemic in most of the tropical and subtropical areas of the world (8, 24). Surveys of dogs from various parts of the United States, Australia and Japan have reported from 1% to 90% of the dogs infected with heartworm (6,9,27,35). In human being, the infection occurs rarely but its consequences can be hazardous (23, 24). According to the report by Güralp (15) canine heartworm infection in Turkey was first detected in 1951. Over the last ten years, it has been reported that *D. immitis* worms and/or microfilariae were frequently seen in dogs in provinces of Elazığ, Ankara, Bursa, Eskisehir, Konya and Van (1, 7, 10, 33, 34, 36, 41). The infection rate varies from 0.06% to 20% (10, 33, 41).

Dirofilariasis is considered to be one of the major cardiopulmonary diseases in dog, and the treatment regimen against the disease is expensive, and complications may develop (6, 19, 30, 32). Therefore, it is necessary to know the heartworm-infected dog's status for determining complications and prognosis of the disease; and therapy method before initiating a program of therapy (6, 18, 31, 37). Beside physical examination and thoracic radiographs, hematologic and serum biochemical variables should be useful to determine disorders associated with heartworm infection (6, 24, 32).

Treatment of heartworm disease in dogs focuses on eliminating *D. immitis* worms mainly with an adulticidal drug (6, 24, 30). It is also necessary to use a microfilaricide before or after adulticidal treatment, because the dogs with circulating microfilariae is a potential threat to uninfected dogs and pathologic changes associated with large numbers of circulating microfilariae occur (6, 18, 24). Chemotherapy in the heartworm infection can be used to two methods. In the commonly preferred method, infected dogs are first treated with thiacetarsamide sodium or melarsomin hydrocholorid, the most effective adulticides (24, 30, 32); three to six weeks later a microfilaricide is used (4, 6, 8, 24, 37). The traditional adulticide, thiarsamide, and a more effective new adulticidal drug, melarsomin, are absent in the market in Turkey. The administration of a microfilaricide such as dithiazanine, levamisole, fenthion, ivermectin and milbemycine (4, 6, 10, 20, 24, 37) is followed by adulticide treatment three to six weeks later. This was reported as the second method by some investigators (6,13, 34). The important complications of adulticide treatment are pulmonary thromboembolism from dying worms and hepatic toxicosis (6, 30-32). Severe liver and kidney pathology, and different adverse reactions are also seen in microfilaricidal treatment (6, 18, 24). The possibility of severe consequences from infection or adulticidal and/or microfilaricidal treatment renders preventive treatment a desirable alternative in endemic areas (6, 18, 24).

Several clinical studies indicated that levamisole had a varying adulticidal efficacy and at the same time highly effect against the microfilariae of *D. immitis* (3, 4, 6, 34). Doramectin is a new avermectin with improved therapeutic spectrum and prolonged activity in comparison with ivermectin (14) and has recently been brought to the market in Turkey. To the authors' knowledge, efficacy of doramectin in heartworm-infected dogs has not been reported until the beginning of the study. Because ivermectin has been shown to be effective in eliminating of *D. immitis* microfilariae (2, 6, 10, 11, 21, 25, 26), we were especially interested in an attempt whether a novel endectocidal drug, doramectin, has an efficacy in dogs with heartworm infections.

The purposes of the study reported here were to evaluate therapeutic efficacy of doramectin and levamisole in naturally acquired *Dirofilaria immitis* infection in dogs, and to determine changes of some hematologic and serum biochemical parameters before and after treatment.

Materials and Methods

A 5 years-old, male German Shepherd dog from the army in Van was admitted to University of Yüzüncü Yıl Veterinary Medical Teaching Hospital with a history of exercise intolerance, coughing, labored respiration, and weight loss. After the physical examination, heartworm infection was suspected and confirmed by the presence of microfilariae in peripheral blood . Two days later, another dead dog from the army was brought to hospital. At necropsy of the dog, 8 adult heartworms were found in the heart and pulmonary arteries. These findings were suggested that other dogs in the army should have been infected with *Dirofilaria immitis*, and their blood samples were examined for presence of microfilariae. Of 15 dogs examined, 11 were found to be infected with *D. immitis*. The heartworm-infected dogs were 9 German Shepherd and 2 Kangal dogs, either sex (10 males an 1 female), 5 to 8 years old and weighed between 27 and 48 kg.

Before treatment, circulating microfilariae were counted for determining periodicity of *Dirofilaria immitis* at 9 a.m. and 18 p.m. collected blood samples for 2 consecutive days. On the 3rd day, after physical examination and blood collection for determining hematologic and serum biochemical

variables, 11 microfilaraemic dogs were divided into 2 treatment groups. Five dogs were given 200 µg/kg of subcutan dose of doramectin (Dectomax*) twice at weekly interval (referred to as Group 1) and the other six dogs were treated with levamisole (Sitrax**) orally at dosage of 10 mg/kg as a daily basis for 14 days (referred to as Group 2). Animals were observed for any possible adverse reactions over a 24 hour period after drug administration. Blood samples were obtained from fasting and resting heartworm-infected dogs for determination of the hematologic and serum biochemical measurements at posttreatment days (PTD) 7, 14 and 28 after initial treatment. Circulating microfilariae were counted at PTD 3, 7, 11, 14, 21 and 28 after initial administration of doramectin and levamisole.

Circulating microfilariae were detected by use of the modified Knott's technique (37). Microfilariae were identified as *Dirofilaria immitis* by their morphological characteristic (17, 27). Microfilarial counts from 2 hemolysed blood samples of 10 µl were counted and averaged (34). Percentage reduction of microfilariae in blood samples was calculated by formul (38). Blood film was stained by use of MayGrünwald-Giemsa stains and the differential leukocyte count was determined (17). Total leukocyte count (WBC), Packed Cell Volume (PCV) and Hemoglobin concentration (Hb) were measured by hemocytometer, microhematocrit and hemiglobincyanid method, respectively (17). Serum creatine kinase (CK), alanine transaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH) activities, and serum total protein concentration (TP) were determined according to the manufacturer's instructions by use of a Technicon RA-XT system. Serum Na and Cl and K concentration were determined, using a Na /Cl/ K analyser, (ISE). One dog in the Group 1 and 1 dog in the Group 2 died 2 and 4 months after initial of treatment, respectively. Necropsy was performed in these dogs and the hearts and lungs were inspected for heartworm.

The student t-Test (28) was used for comparison of the data before and after drug administrations and for comparison of pretreatment data between the groups.

Results

Eleven of the 15 or 73.3% of the dogs examined by using modified Knott technique were found to be infected with *D. immitis*.

Before treatment, 11 heartworm-infected dogs had an average rectal temperature of 39.1°C (38.5-40.0) and an average heart rate of 98.7 beats/min. (84-140). Chief signs in most of the 11 heartworm-infected dogs were weakness, decreased performance, a spontaneous or elicited cough easily by tracheal pressure, dyspnoea, exaggerated or harsh vesicular sounds, cardiac insufficiency such as holosystolic heart murmur, accentuating the second heart sound and arrhythmia. Some dogs showed no particular signs. Although a reduction in severity of signs was noticed by the physical examination after treatment, signs did not disappear. Adverse reactions associated with doramectin administration were observed in 1 dogs with high microfilariae count, and included vomiting, lethargy and anorexia. The adverse reactions occurred within 24 hr after first doramectin administration, and recovery took place 2 to 12 hr after onset of the reaction without treatment. Furthermore, a fleeting painful and an itching reaction were observed in 2 dogs at time of doramectin injection. A transient and slight vomiting, diarrhoea, anorexia and weakness caused by levamisole were observed in 2 dogs after first or second treatment. These adverse reactions disappeared within 24 hr. after drug administration without special treatment. Two and 4 months after initiating treatment, one dog administered doramectin and one other dog given levamisole died. At necropsy of these dogs 24 and 32 heartworms were found in heart and pulmonary artery. The main findings were enlargement of the right ventricle, the main pulmonary artery and thromboembolic area in the left caudal lung lobe.

Laboratory test results in both groups before and after treatment were outlined in the Table 1-4. All data in the Tables were expressed as mean ± SEM and range (minimum-maximum). Pretreatment data in general and both groups are presented in Table 1. Mean microfilaria count in blood samples collected at 18 p.m. was significantly ($p<0.01$) higher than in blood samples collected at 9 a.m. The pretreatment data for dogs of Group 1 were not significantly different from those of Group 2. Although the difference in mean microfilaria count between Group 1 and Group 2 was not significant, all dogs in the Group 1 had less microfilariae than those of Group 2. PCV, Hb and CK values in the 11 heartworm-infected dogs were found to be increased as compared with normal ranges of adult dogs. Mean lymphocyte counts in general and both groups showed a slight lymphopenia (<1000 lymphocytes/µl). In some dogs, there were a leukopenia (<6000 WBC/µl), a neutropenia (<3000 neutrophils/µl) and/or an eosinophilia (>1250 eosinophils/µl). The mean absolute eosinophil count was normal, but the mean relative number of eosinophils (13.8%) in general was high. Monocyte and basophil counts

* Dectomax, Pfizer Ilaçları A.Ş., İstanbul
** Sitrax, Doğu İlaç Fabrikası, İstanbul

indicated no changes. The serum biochemical variables, except CK activity, were almost within normal limits. In the doramectin-treated dogs (Table 2), comparison of data before and after treatment indicated that there were a transient, but significant decrease in PCV and Hb values, eosinophil count, serum CK activity, serum TP and Cl concentrations and a transient, but significant increase in neutrophil and lymphocyt counts and serum K concentration after doramectin application. Neutrophil and lymphocyt counts were increased at PTD 7 and 14, respectively. The other variables did not change significantly.

In the levamisole-treated dogs (Table 3), significant differences that were found between pre- and posttreatment measurements included PCV and Hb values, eosinophil, neutrophil and lymphocyte counts, serum CK and ALT activities, and serum TP, Na, Cl and K concentrations. After levamisole administrations, PCV and Hb values, eosinophil count, serum CK activity and serum TP and Cl concentrations decreased, neutrophil and lymphocyte counts, serum ALT activity and serum Na and K concentrations increased, transiently. Changes in the other variables were not significant.

In Group 1 (Table 4), the microfilaria count was decreased by 6.5 % to 13.1 % of the pretreatment value during the 7 days after doramectin application. The microfilaria count remained stable for the second week and tended to increase gradually thereafter without returning to the pretreatment value. In Group 2, the microfilaria count was decreased by 8.1 % to 12.4 % of the pretreatment value during the 7 days after levamisole administration. In contrast to Group 1, the microfilaria count steadily decreased until day 14. From day 14, the microfilaria count tended to increase gradually without returning to the pretreatment value.

Table 1-Laboratory test results in general and both groups before treatment

Variable	General (n=11)		Group 1 (n=5)	Group 2 (n=6)
PCV(%)	58.8±1.1 (56-65)		58.8±1.9 (56-65)	58.8±1.3 (57-63)
Hb(g/dl)	21.3±0.4 (19.7-23.50)		20.7±0.5 (19.7-22.5)	21.7±0.6 (20.4-23.5)
WBC (cells/ µl)	6750±602 (3675-9450)		6800±1153 (3675-9275)	6708±664 (5200-9450)
Eosinophil count (cells/µl)	931±98 (520-1456)		944±199 (546-1456)	904±87 (520-1134)
Neutrophil count(cells/µl)	4731±458 (2315-7182)		4741±820 (2315-6400)	4724±566 (3376-7182)
Lymphocyte count(cells/µl)	915±90 (455-1391)		904±170 (455-1299)	868±57 (686-1040)
CK (IU/L)	219.4±17.5 (125-298)		239.0±18.8 (183-298)	203.0±27.8 (125-288)
ALT (IU/L)	35.2±3.5 (15-53)		33.6±6.5 (15-53)	36.5±4.0 (25-52)
AST (IU/L)	30.9±1.8 (21-40)		33.6±3.8 (22-43)	30.3±2.4 (21-38)
LDH (IU/L)	122.4±13.2 (34-196)		147.6±18.4 (82-196)	101.3+14.7 (34-136)
GGT (IU/L)	4.3±0.5 (0-7)		3.4±1.0 (0-6)	5.0±0.5 (4-7)
TP (g/dl)	7.4±0.2 (6.4-8.4)		7.4±0.7 (6.5-8.4)	7.3±0.3 (6.4-8.0)
Na (mmol/L)	145.9±0.4 (142.6-148.2)		146.4±0.1 (146.1-146.8)	145.5±0.8 (142.6-148.2)
Cl (mmol/L)	115.2±0.8 (110.0-119.6)		116.4±1.3 (112.1-119.6)	114.3±0.9 (110.0-116.0)
K (mmol/L)	4.5±0.1 (4.0-4.8)		4.5±0.1 (4.2-4.8)	4.4±0.1 (4.0-4.8)
Microfilaria count	9 a.m.	18 p.m.	18 p.m.	18 p.m.
(number/10µl)	27.7±8.9 (7-99)	62.1±17.7** (21-197)	52.0±24.6 (21-150)	70.8±26.8 (23-197)

*** Significantly different (p< 0.01) from the microfilaria count at 9 a.m.*

Table 2. Hematologic and serum biochemical variables in the Group 1 before and after treatment

Variable	Before treatment	Days after treatment 7	14	28
PCV(%)	58.8±1.9 (56-65)	56.2±1.6* (53-62)	55.4±1.2* (54-60)	60.2±0.8 (62-68)
Hb(g/dl)	20.7±0.5 (19.7-22.5)	18.6±0.4* (18.1-21.4)	19.6±2.5 (15.3-22.0)	20.8±0.5 (19.4-22.3)
WBC (cells/ µl)	6800±1153 (3675-9275)	7570±748 (5350-9115)	7955±989 (5670-11525)	7895±856 (6100-10450)
Eosinophil count (cells/µl)	944±199 (546-1456)	612±131* (375-951)	383±49** (284-536)	1032±232 (580-1640)
Neutrophil count (cells/µl)	4741±820 (2315-6400)	5740±687* (3745-7209)	5669±729 (4200-8298)	5430±740 (3400-9380)
Lymphocyte count (cells/µl)	904±170 (455-1299)	1018±105 (695-1275)	1678±223* (965-2305)	1165±120 (765-1432)
CK (IU/L)	239.0±18.8 (183-298)	138.8±4.0** (130-150)	207.4±13.8 (173-256)	170.2±12.1* (152-217)
ALT (IU/L)	33.6±6.5 (15-53)	43.6±10.2 (15-78)	45.0±9.1 (16-60)	43.6±7.6 (21-64)
AST (IU/L)	33.6±3.8 (22-43)	33.8±5.0 (19-46)	34.4±3.4 (23-42)	37.2±6.4 (23-60)
LDH (IU/L)	147.6±18.4 (82-196)	113.8±38.0 (64-265)	185±48.3 (67-340)	180.4±34.2 (89-268)
GGT (IU/L)	3.4±1.0 (0-6)	4.6±0.9 (2-6)	5.2±0.5 (4-6)	4.9±1.2 (4-6)
TP (g/dl)	7.4±0.7 (6.5-8.4)	6.7±0.2** (6.2-7.3)	7.1±0.3 (6.5-.8)	6.9±0.2* (6.3-7.4)
Na (mmol/L)	146.4±0.1 (146.1-146.8)	148.0±0.8 (46.2-150.7)	146.1±0.6 (144.8-148.4)	146.4±0.4 (145.2-147.6)
Cl (mmol/L)	116.4±1.3 (112.1-119.6)	111.3±1.6** (107.3-114.7)	109.6±3.1** 106.5-113.9)	115.5±0.6 (13.7-116.9)
K (mmol/L)	4.5±0.1 (4.2-4.8)	4.9±0.2** (4.5-5.4)	4.4±0.1 (4.2-4.8)	4.3±0.1 (4.1-4.7)

** and ** Significantly (p< 0.05 and p< 0.01 , respectively) different from pretreatment value.*

Discussion

Eleven of the 15 or 73.3 % of the dogs examined in this study were found to be infected with heartworm. The high amount of infection in Van was not surprising. The ages, breed and sexes of the dogs; and especially their life style, living area and the absence of preventive measure can be considered to possible reasons for the high amount of infection. The microfilaraemic 11 dogs were 5 to 8 years old, large breed and 10 males and 1 female. These findings were similar to the report by Calvert (6). Calvert (6) reported that the mean age of heartworm-infected dogs diagnosed by microfilaremia and necropsy was six to seven years, large dogs were infected most frequently, and male dogs had higher infection rates than females. Because *D. immitis* depends on the mosquito as an intermediate host to complete its life cycle, high mosquito density is generally associated with heartworm (8, 24). Mosquitoes are widely seen around Van Lake. Therefore, the dogs that have been used in military work for 5 to 6 years in Van province, had largely exposure to mosquitoes. Add to this, the dogs had not been given medication for prevention of heartworm disease.

Before treatment, main clinical signs were similar to those that have been described (6, 18, 24). The slight and transient adverse reactions caused by administration of doramectin were observed in 1 dogs with high concentration of circulating microfilariae and resembled those in heartworm infected dogs treated with ivermectin (6). Levamisole-related adverse reactions in 2 dogs observed in this study were partly similar to those that have been reported (4, 6, 24, 34). When the number of circulating microfilariae in blood samples collected at 9 a.m. was compared with the number at 18 p.m. , more microfilariae has been found in the early evening. This result corresponded with the other reports (4, 17, 18, 24).

Hematologic and serum biochemical variables in heartworm disease did not reveal consistent abnormalities, with the except for circulating microfilariae (6, 18). Dependent on severity of the disease, among other things;

Table 3. Hematologic and serum biochemical variables in the Group 2 before and after treatment

Variable	Before treatment	Days after treatment 7	14	28
PCV (%)	58.8±1.3 (57-63)	52.2±0.6** (50-54)	52.0±2.2* (49-63)	60.2±0.8 (58-62)
Hb (g/dl)	21.7±0.6 (20.4-23.5)	18.9±0.7** (17.0-21.8)	18.2±1.4** (16.2-20.3)	20.2±0.6 (18.5-22.3)
WBC (cells/ µl)	6708±664 (5200-9450)	7508±950 (4575-11025)	8658±1094 (5750-12900)	8365±1040 (6200-12440)
Eosinophil count (cells/ µl)	904±87 (520-1134)	543±63** (366-742)	426±113* (109-903)	980±140 (640-1365)
Neutrophil count (cells/ µl)	4724±566 (3376-7182)	5788±809* (3384-8930)	6491±1015 (3738-10062)	6132±1085 (4165-9865)
Lymphocyte count (cells/ µl)	868±57 (686-1040)	967±60 (732-1113)	1422±99** (1043-1677)	1201±115 (1185-1496)
CK (IU/L)	203.0±27.8 (125-288)	147.8±23.1* (92-223)	219.5±24.4 (141-296)	149.5±5.6 (133-175)
ALT (IU/L)	36.5±4.0 (25-52)	200.0±45.7* (70-371)	418.8±161.4 (54-1030)	58.0±18.0 (25-143)
AST (IU/L)	30.3±2.4 (21-38)	64.0±13.0 (31-107)	64.2±18.8 (27-132)	32.5±2.6 (22-40)
LDH (IU/L)	101.3±14.7 (34-136)	99.5±19.1 (62-192)	172.3±46.0 (73-360)	182.3±33.5 (66-275)
GGT (IU/L)	5.0±0.5 (4-7)	4.8±0.7 (3-8)	5.0±0.9 (3-8)	6.7±0.5 (6-9)
TP (g/dl)	7.3±0.3 (6.4-8.0)	6.5±0.2** (6.1-7.3)	7.1±0.2 (6.6-7.9)	7.2±0.2 (6.6-7.6)
Na (mmol/L)	145.5±0.8 (142.6-148.2)	147.5±1.0* (144.1-150.6)	146.4±1.1 (142.2-148.9)	144.8±0.9 (141.8-147.8)
Cl (mmol/L)	114.3±0.9 (110.0-116.0)	112.5±1.5 (106.2-115.6)	110.1±0.8** (108.6-113.8)	114.2±0.7 (112.9-116.9)
K (mmol/L)	4.4±0.1 (4.0-4.8)	4.3±0.1 (3.9-4.8)	4.8±0.1** (4.5-5.3)	4.5±1.4 (4.1-4.9)

** and ** Significantly (p< 0.05 and p< 0.01, respectively) different from pretreatment value.*

Table 4. Effect of doramectin (Group 1) and levamisole (Group 2) against microfilariae of *D. immitis*.

		Before treatment	Days after treatment 3	7	11	14	21	28
Group 1	Microfilaria count (num/10µl)	52.0±24.6 (21-150)	6.8±2.3* (1-15)	3.4±0.8** (1-6)	5.8±1.5** (1-10)	3.4±0.7** (1-5)	8.5±1.1* (2-12)	10.2±2.2* (5-15)
	RM (%)		86.9	93.5	88.8	93.5	83.6	80.4
Group 2	Microfilaria count (num/10µl)	70.8±26.8 (23-197)	8.8+2.5*** (2-19)	5.7±1.5** (1-11)	3.5±0.9** (1-6)	3.7±0.3*** (2-4)	9.3±1.5** (5-15)	11.4±4.3** (5-20)
	RM (%)		87.6	91.9	95.1	94.8	86.7	83.9

RM= Reduction in Microfilaria count

, ** and * Significantly (p< 0.05 , p<0.01 and p< 0.001 , respectively) different from pretreatment value (According to geometric means)*

anaemia, neutrophilia, eosinophilia, mild-moderate elevations in serum transaminase and CK levels, and elevations in the serum beta and gamma globulin fractions may all be seen in heartworm disease (6, 18, 19). In the present study, PCV and Hb values before treatment (Table 1.) were found 58.8% (56-65) and 21.3 g/dl (19.7-23.5), respectively. Normal PCV and Hb values in adult dogs are 45% (37-55) and 15 g/dl (12-18), respectively (8, 17). In contrast to anemia that is observed 10 to 50% of dogs with heartworm disease (6), the increase in PCV and Hb values detected pretreatment can be attributed to an appropriate compensatory response to chronic hypoxia due to cardiopulmonary insufficiency (5, 17, 18). Slight, but significant decreases in PCV and Hb after treatment (Tables 2 and 3) may indicate transient haemodilution, haemolysis or both (5, 17, 20). Except for the slight lymphopenia and some individual changes, the leukograms (Table 1), compared with reference range in adult dogs (5, 8, 17), were not effected by the infection. These findings were almost similar to those reported by some investigators (8, 12, 29, 30) but did not cover the reports by others (5, 6, 17, 18). Reasons for this disparity may be related to stage of the infection (5, 6). Before treatment, decreased lymphocyte count and individual eosinophilia may be attributed to a stress response and a response to parasitic antigen, respectively (5, 17). A transient, but significant increase in neutrophil count and a transient, but significant decrease in eosinophil count after treatment (Tables 2 and 3) can be considered to indicate possible increase in catechlolamine and corticosteroid concentrations accompanying stress, and inflammatory reactions (5, 8, 17). In adult dogs, the reported normal values of serum CK activity extended to 97 IU/L (22) , 126 IU/L (19) and 200 IU/L (39). Increased activity of serum CK in heartworm disease was found before treatment also in most of the dogs in this study as reported by others (19). As reported by Kitagawa et. al. (19), CK release from heartworms and from an injured myocardium or skeletal muscles might be possible reasons for increased serum CK activity also in most of the 11 microfilaramic dogs (Table 1). The other serum enzyme activities monitored before treatment were generally within or near to normal ranges (22, 39). This result supported the findings in heartworm disease as observed by others (20, 31). A partial improvement may be contributory to a slight, but significant decrease in serum CK activity in both groups after treatment (Tables 2 and 3). In contrast to doramectin, levamisole seemed to increase serum ALT activity. This had been previously observed and related to an increased inflammatory reactio (6, 16, 20). Hyperproteinaemia (TP >8.0 g/dl) noticed in 2 dogs before treatment and a slight, but significant decrease in TP value after treatment (Tables 2 and 3) can be resulted from an increase in beta and gamma globulins and a pharmacologic action of the compounds, respectively (6, 17, 18, 20). After treatment, serum Cl and K in the Group 1 and serum Na, Cl and K in the Group 2 changed significantly (Tables 2 and 3). The changes can be related to a complex of pharmacologic actions of the drugs or circadian rhythm. However, changes in these values were slight and values were generally within or near to the reference ranges (5, 8, 39).

In this study, doramectin could be given only 5 dogs with less with concentration of circulating microfilariae, because we have been unable to find any reports on the effects of doramectin in canine heartworm infection, ivermectin was known to cause adverse reactions in dogs with high microfilaria count (6) and the dogs were in military use.

In the Group 1, the decrease in circulating microfilariae after treatment indicated that doramectin at a dosage 200 µg/kg had a microfilaricidal efficacy. Reductions in the number of microfilariae ranged from 86.9 % to 93.5 % during the first 2 weeks after treatment (Table 4). There was yet no report in which the efficacy of doramectin has been shown in canine heartworm infection to date. Therefore, microfilaricidal efficacy of doramectin noticed here could not be drawn a comparison. However, from these results it can be considered that doramectin is at least as effective as a commonly used microfilaricide, ivermectin. Findings in several studies showed that efficacy of ivermectin against microfilariae of *D. immitis* depended on dosage, age of microfilariae and therapy method, and varied from 64% to 100% (2, 6, 10, 11, 21, 24, 26). Allan et. al. (2) reported that efficacy of ivermectin in equal dosage were 97.2% and 63.8% against 30 and 45 day-old microfilariae from induced infections, respectively. The administration of ivermectin after adulticidal treatment eliminated microfilariae completely, while this could not be achieved in adulticide untreated dogs with heartworm infections (21, 25). The microfilaricidal efficacy of doramectin differed from ivermectin of some studies in which 100% efficacy had been achieved (11, 21, 25, 26). Reasons for the different effect were possible connected with age of microfilariae and therapy method.

In the Group 2 (Table 4), the decrease in microfilaria count varied from 87.6 % to 95.1% during first the 2 weeks after levamisole treatment and this finding corresponded with the reports by others (3, 4, 6, 8, 34). It has been reported that levamisole eliminates microfilariae in about 90 % of affected dogs when administered at a daily dosage of 10 to 11 mg/kg for one to two weeks (6).

One the basis of microfilaricidal efficacy, levamisole appeared to be more effective in eliminating microfilariae than doramectin was (Table 4). The difference in reduction of microfilarial count between doramectin and levamisole can be attributed to the partial adulticidal efficacy of levamisole. Although the number of microfilariae was apparently reduced after doramectin and levamisole treatment, it was not attainable to eliminate the organisms completely. Incomplete elimination of circulating microfilariae until day 14 and theirs gradually increase after that (Table 4.) can be speculated as the presence of gravid female worms. Calvert (6) also reported on difficulty to clear circulating microfilariae when gravid female worms were present and on often persistence of low concentration of microfilariae, even though all adult worms have been eliminated.

It has been reported that serum heartworm antigen level correlates significantly with adult worms counts, and its measurement is a good indicator to monitoring of success of a adulticidal drug in *Dirofilaria immitis* infections (30, 31, 40). In the present study, this test and arteriography (30) for monitoring of adulticidal efficacy of doramectin and levamisole could not be implemented. However, no apparently improvement in main clinical findings after treatment and the presence of live adult heartworms at the necropsy of one dog from each group revealed that levamisole as well as doramectin had an unsatisfactory (levamisole) or not any therapeutic efficacy against *D. immitis* worms.

In conclusion, this study showed that canine heartworm infection in Van may be widely, also doramectin had a highly microfilaricidal efficacy, and the infection and the treatments induced changes in some hematologic and serum biochemical variables. Owing to increased dog population and movement of dogs in the last years, canine heartworm infection requires more consideration in differential diagnosis of cardiopulmonary diseases. It is necessary to put an effective adulticidal drug on the market for successful treatment and by this means prevention of the infection.

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