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Assessment of Clinical Outcome in Dogs with Naturally Infected with *Dirofilaria immitis* after American Heartworm Society Protocol *vs* Slow Kill Method

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Abstract : Heartworm disease (HWD) in dogs is a life-threatening mosquito-borne disease resulting in right-sided congestive heart failure and inflammatory pulmonary disease. Due to complications from adulticidal therapy with melarsomine, slow kill protocol either with preventive dose of ivermectin or combined with doxycycline has been proposed for an alternative adultcidal therapy in dogs with HWD. Therefore, this study evaluated the clinical outcome of adultcidal therapy in dogs with class II stage of HWD after treating either American Heartworm Society (AHS) or slow kill protocol for 10 months. Clinical outcome after therapy was evaluated by clinical, radiographic and echocardiographic examination along with hematology before (D0) and after therapy (D300). Although clinical signs associated with HWD were all resolved after therapy in both groups, the infection was not cleared out 67% of dogs treated by slow kill protocol at the end of therapy. Furthermore, pulmonary arterial flow of acceleration time to ejection time ratio (AT/ET) and the right pulmonary artery distensibility index (RPADI) have been firstly used for detecting pulmonary hypertension in this study group. The pulmonary hypertension was more common in dogs with mild clinical signs, although tricuspid and pulmonary regurgitation were not detectable in most dogs in this study. Our study findings suggested that the slow kill protocol might not be efficacious enough to clear out HWD in dogs and more attention on the presence of pulmonary hypertension might be necessary for effective management of HWD in dogs.

Key words **:** heartworm, dirofilariasis, melasormine, AHS guideline, slow kill.

Introduction

Heartworm disease (HWD) in dogs is a mosquito-borne infectious disease caused by *Dirofilaria immitis* and is occurring worldwide including Korea. HWD is primarily a cardiopulmonary disease resulting in right-sided congestive heart failure with pulmonary hypertension and allergic pulmonary inflammation with thromboembolism in dogs (13). Clinical signs related to HWD are closely associated with worm burden. Treatment is differed by patient age, worm burden, and severity of clinical signs. Conservative microfilaricidal, antimicrobial and anti-inflammatory therapy is generally recommended in older dogs or dogs with heavy worm burden and/or dogs having secondary congestive heart failure, pneumonia and circulatory disorders. Interventional worm removals are necessary in dogs with caval syndrome or heavy worm burden (8).

Although currently available adulticidal drugs are effective for killing adult worms, it can often increase risks of mortality occurred from the process of clearance of dead and dying adult worms (7). According to current guidelines from the American Heartworm Society (AHS), a three-step melarsomine injection protocol was recommended in dogs with class I-III stage of HWD, and permits for a more staged elimination of the heartworm. Unavailability of melarsomine on the market and concerns of post-adulticidal complications from melarsomine leaded to develop alternative adulticide treatments (1). Recent studies found long-term administration of ivermectin with preventive dose (slow kill method) could kill adult worms in both experimentally- and naturally-infected dogs (10,13,17). Furthermore, one other study combined ivermectin/doxycycline protocol found that clearance of infection in 75% of naturally infected dogs after 10 months of therapy (4). However, slow kill method could lead the resistance of macrocyclic lactones and more serious complications during treatment period (17), and thus the current AHS guidelines do not recommend the slow kill in dogs with HWD. There has not yet been direct comparison study for assessing the clinical outcome between these two protocols. Therefore, the aim of this study was to evaluate clinical outcome of adultcidal therapy in dogs with class II stage of HWD after treating either AHS or slow kill protocols for 10 months.

Materials and Methods

Study population

Twelve mixed dogs infected with HWD were enrolled in

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this study. All were brought from private animal shelter. The owner of private animal shelter gave their consent for their dogs to participate in this study. Heartworm infection in this study population was diagnosed using a commercial ELISA test kit (SNAP® 4Dx® Plus Test, Idexx Laboratory, USA), as instructed by manufacturer's manual. The presence of microfilariae was further evaluated by a modified Knott's test. Only dogs with the class II stage of HWD were selected, based on clinical, radiographic and echocardiographic exams: Class II having mild clinical signs (i.e., occasional cough with or without exercise intolerance) with abnormal findings in radiographic (i.e., mild pulmonary infiltration and pulmonary arterial dilation), and echocardiographic examinations (i.e., dilation of main pulmonary artery without interventricular septal flattening) described in 2014 AHS guideline. The dogs were divided into two groups and treated by either AHS protocol (n = 6), or slow kill protocol (n = 6) for 10 months. In the AHS protocol, all dogs were administered with preventive dose of ivermectin (6-10 μg/kg, PO, monthly for 2 months, Heartguard, Merial, USA) and doxycycline (10 mg/kg, PO, q12hr for 4 weeks, Vibramycin, Pfizer, Seoul, Korea) prior to administration of the adulticide melarsomine dihydrochloride. Three doses of melarsomine (Immiticide®, Merial, USA) were injected as instructed from manufacturer. A single injection at 2.5 mg/kg at deep intramuscular injection into the belly of the epaxial lumbar muscles (between L3 and L5), followed 1 month later by two injections of 2.5 mg/kg administered 24hr apart. In slow kill protocol, all dogs were administered with preventive dose of ivermectin (6-10 μg/kg, PO, bi-weekly, Heartguard, Merial, USA) and doxycycline (10 mg/kg, PO, q12hr for 4 weeks, Vibramycin, Pfizer, Seoul, Korea) for 9 months after initiation of therapy. Any steroid and anticoagulant therapies were not combined, to minimize the influence on clinical outcome during study period.

Assessment of clinical outcome

The clinical outcome was evaluated before (D0) and 10 months (D300) after the initiation of therapy. Dogs were submitted to complete clinical examination, laboratory test, thoracic radiography and echocardiography at the initiation (D0) and the termination (D300) of therapy. Scoring criteria of clinical outcome were summarized in Table 1. Thoracic radiographs were taken at right lateral and ventrodorsal projection. Echocardiographic measurements were performed with an ultrasound machine (X-300, Simens, Germany) with a 3-9 MHz phase-array transducer. For echocardiographic assessment, pulsed wave (PW)-spectra signals for calculation of pulmonary arterial flow of ejection time (ET) and acceleration time (AT) were acquired from the right parasternal short axis view of the pulmonary artery with the sample volume at the valve level. The right pulmonary artery distensibility index (RPADI) was measured as described in Venco *et al* (18). Briefly, the minimum diastolic (RPADd; usually at the Q wave) and maximum systolic (RPADs; usually coinciding with the largest T wave deflection or early-to-midsystole) internal diameter of the right pulmonary artery (PA) was quantified at the same location of the right PA, using a right parasternal short axis of PA on 2D echocardiography. The RPADI was calculated using the following formula: RPADI = (RPADs − RPADd]/RPADs) × 100. CW-spectra of tricuspid regurgitation, when present, were acquired from the left apical cranial four-chamber views. The AT/ET ratio and tricuspid regurgitation velocity (TRV) were measured off-line by a single observer (Hyun) blinded to dog identification, clinical data and radiographic results. Blood samples were drawn from the cephalic vein of each animal for measurements of complete blood cell counts (ProCyte Dx Hematology Analyzer, Idexx, USA).

Statistical analysis

For each time point during the study (D0 and D300), mean scores from all dogs for clinical exam, thoracic radiography and echocardiography were determined. Obtained values were compared by student *t*-test (SPSS for Microsoft Windows, version 11.0) and a p-value of < 0.05 was considered significant.

Results

The age of dogs enrolled in this study was unable to be determined, although all dogs were matured. The body weight of dogs in AHS group was 6.3 ± 1.3 kg (1 male and 5 female), while that in slow kill group was 10.1 ± 6.0 kg (2 male and 4 male). Female was predominant in both study group, while slow kill group was ~ 2 times heavier than AHS group.

Therapeutic success based one HWD antigen test was 2/6 (33%) in slow kill group and 6/6 (100%) in AHS group, respectively, although microfilaremia was not found in both group. Clinical signs associated with HWD were all resolved in both groups at D300. Score for clinical signs was remarkably reduced in both groups (Table 2). Score for thoracic radiographic signs was also reduced in both groups, although 1/6 dogs in slow kill group and 2/6 dogs in AHS group still had radiographic signs at D300 (Table 2). Score for echocardiographic signs was all reduced in both groups. Echocardiographic indices for HWD were all returned to normal reference range in both groups.

Mean ± standard deviations of ET and AT were 121 ± 6 and 41 ± 7 msec (AT/ET 0.34 ± 0.06), respectively in slow kill group at D0, while those were 123 ± 10 and 58 ± 9 msec (AT/ET 0.47 ± 0.06), respectively, at D300. In contrast, mean ± standard deviations of ET and AT were 122 ± 11 and 40 ± 6 msec (AT/ET 0.33 ± 0.06), respectively, in AHS group at D0, while those were 123 ± 10 and 57 ± 12 msec, respectively, at D300 (AT/ET 0.46 ± 0.08; Table 2). The AT/ET ratio of all dogs in both groups were below reference range (< 0.42) at D0, while 1/6 dogs in slow kill group and 1/6 dogs in AHS group remained below reference range at D300. Mean ± standard deviation of RPDAI was 24 ± 5 in slow kill group at D0, while that was 47 ± 6 at D300. In contrast, mean ± standard deviation of RPDAI was 24 ± 5 in AHS group at D0, while that was 54 ± 11 at D300 (Table 2). The RPADI of all dogs in both groups was below reference range (< 40) at D0, while 1/6 dogs in slow kill group and 1/6 dogs in AHS group remained below reference range at D300. TR was noticed in 3/6 dogs in slow kill group and 3/6 dogs in AHS group. After therapy, 1/6 dogs in AHS group still had TR at D300, although no dogs showed hemodynamically sig-

Table 1. Scoring criteria for assessment of clinical outcome

CLINICAL SIGNS			
0	1	2	3
Asymptomatic	Exercise intolerance, cough, Thin body condition	Constant fatigue, persistent cough, dyspnea, weight loss	Right-sided congestive heart failure, jugular pulses, ascites
THORACIC RADIOGRAPHY			
0	1	2	3
Normal	Mild unstructured interstitial lung pattern in the caudal lobe	More diffuse and uniform unstructured interstitial lung pattern	Diffuse pulmonary densities, signs compatible with thromboembolism/pneumonia
ECHOCARDIOGRAPHY			
0	1	2	3
Normal (AT > 64 ms; AT/ET ratio > 0.42), RPADI > 40	Slight alterations (AT/ET = 0.42-0.26 ms), RPADI 25-40	Moderate alterations (AT ≤ 45 ms; AT/ET ≤ 0.25), RPADI 10-25	Marked alterations (moderate/severe pulmonary hypertension: TRV > 3.57 m/s), RPDAI < 10

AT; Acceleration time, ET; Ejection time, RPADI; Right pulmonary artery distensibility index, TRV; Tricuspid valve regurgitation velocity.

Table 2. Clinical outcome in heartworm infected dogs treated after either slow kill or AHS method

		Clinical score	Radiology score	M-filaria	HW-Ag	ET msec	AT msec	AT/ET	RPDAI	TR	RBC M/uL	WBC K/uL	Neutro K/uL	Lymph K/uL	Eosino K/uL
Slow kill (n = 6)															
DAY 0	Mean	1.3	1.3	6/6	6/6	121	41	0.34	24.3	3/6	5.8	8.7	5.2	1.5	1.4
	SD	0.5	0.5			6	7	0.06	4.8		1.1	3.9	2.9	0.4	0.3
DAY 300	Mean	0.2*	0.2*	0/6*	4/6	123	58*	0.47*	46.7*	0/6*	7.1	12.4	7.6	2.4	1.5
	SD	0.4	0.4			10	9	0.06	6.3		1.1	4.8	3.5	1.0	1.0
AHS (n = 6)															
DAY 0	Mean	1.3	1.3	6/6	6/6	122	40	0.33	23.8	3/6	7.2	9.7	5.6	1.8	1.3
	SD	0.5	0.5			11	6	0.06	5.0		0.4	1.0	0.6	0.7	0.1
DAY 300	Mean	0.2*	0.3*	0/6*	0/6*,#	123	57*	0.46*	53.8*	1/6*	7.4	7.9	4.7	1.6#	0.8#
	SD	0.4	0.5			10	12	0.08	10.6		0.9	1.7	1.1	0.4	0.4

*. $P < 0.05$ DAY 0 *vs* DAY 300, #. $P < 0.05$ Slow kill *vs* AHS

AHS; American Heartworm Society, AT; Acceleration time, ET; Ejection time, RPADI; Right pulmonary artery distensibility index, TR; Tricuspid valve regurgitation velocity, M-filaria; modified Knott test for microfillaria, HW-Ag; heartworm antigen test.

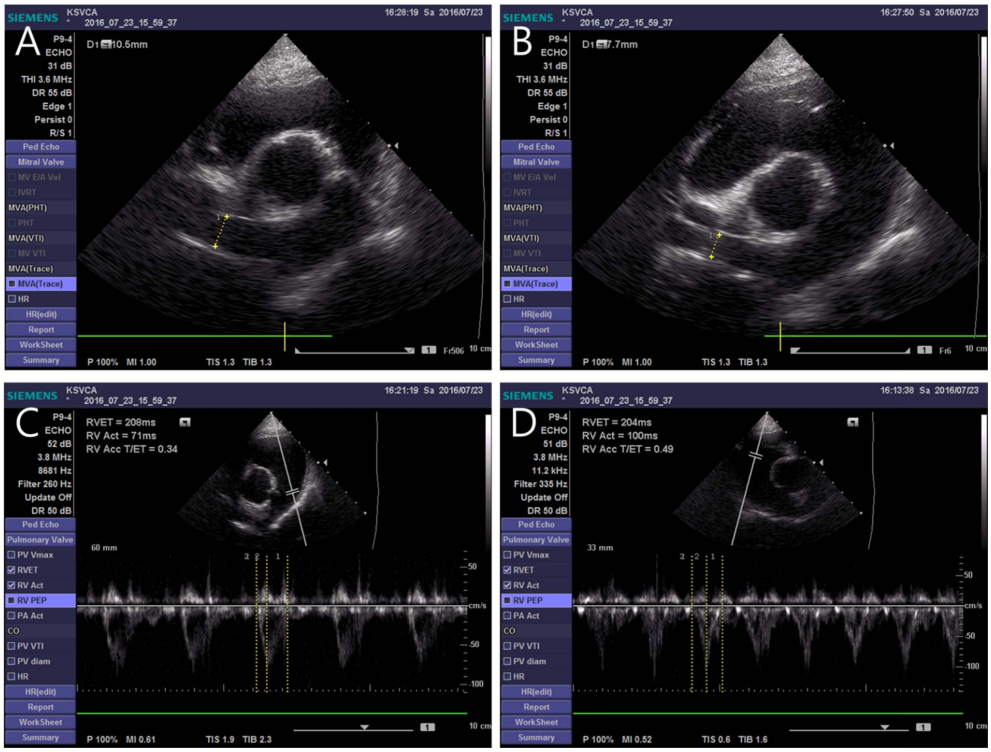

Fig 1. Echocardiographic assessment in dogs with heartworm disease. Measurement of right pulmonary distensibility index in systole (A) and diastole (B), ejection time/accelation time ratio of pulmonary flow in affected (C) and normal (D) dogs.

nificant TR (> 2.8 m/s; Table 2).

Anemia was noticed in 2/6 in slow kill group (5.8 ± 1.1 × 10 M/μL) and 0/6 in AHS group (7.2 ± 0.4 × 10 M/μL) at D0, respectively, while that was in 1/6 in slow kill (7.1 ± 1.1 × 10 M/μL) and 0/6 in AHS group (7.4 ± 0.9 × 10 M/μL) at D300, respectively (Table 2). Leukocytosis was noticed in 0/6 in slow kill group (8.7 ± 3.9 × 10 K/μL) and 0/6 in AHS group (9.7 ± 1.0 × 10 K/μL) at D0, respectively, while that was in 1/6 in slow kill (12.4 ± 4.8 × 10 K/μL) and 0/6 in AHS group (7.9 ± 1.7 × 10 M/μL) at D300, respectively (Table 2). Leukocytosis was noticed in 0/6 in slow kill group (8.7 ± 3.9 × 10 K/μL) and 0/6 in AHS group (9.7 ± 1.0 × 10 K/μL) at D0, respectively, while that was in 1/6 in slow kill (12.4 ± 4.8 × 10 K/μL) and 0/6 in AHS group (7.9 ± 1.7 × 10 M/μL) at D300, respectively (Table 2). Neutrophilia was noticed in 1/6 in slow kill group (5.2 ± 2.9 × 10 K/μL) and 0/6 in AHS group (5.6 ± 0.6 × 10 K/μL) at D0, respectively, while that was in 1/6 in slow kill (7.6 ± 3.5 × 10 K/μL) and 0/6 in AHS group (4.7 ± 1.1 × 10 M/μL) at D300, respectively (Table 2). Lymphocytosis was noticed in 0/6 in slow kill group (1.5 ± 0.4 × 10 K/μL) and 0/6 in AHS group (1.8 ± 0.7 × 10 K/μL) at D0, respectively, while that was in 1/6 in slow kill (2.4 ± 1.0 × 10 K/μL) and 0/6 in AHS group (1.6 ± 0.4 × 10 M/μL) at D300, respectively (Table 2). Eosinophillia was noticed in 4/6 in slow kill group (1.4 ± 0.3 × 10 K/μL) and 6/6 in AHS group (1.3 ± 0.1 × 10 K/μL) at D0, respectively, while that was in 3/6 in slow kill (1.5 ± 1.0 × 10 K/μL) and 0/6 in AHS group (0.8 ± 0.4 × 10 M/μL) at D300, respectively (Table 2). Leukopenia was noticed in 2/6 in slow kill group and 0/6 in AHS group at D0, respectively, while no dogs showed leukopenia in both groups at D300 (Table 2). No dogs in both groups showed neutropenia, lymphopenia and eosinopenia during test period.

Discussion

Due to complications (e.g., pulmonary thromboembolism, pulmonary intense pro-inflammatory reactions) from adulticidal therapy with melarsomine (7), the 2002 AHS guideline proposed the monthly administration of preventive dose of ivermectin (6 μg/kg could be an alternative adulticidal protocol for dogs with HWD (11). Several studies also found that adultcidal therapy with preventive dose of ivermectin might need at least 2-3 years to accomplish complete efficacy (10,17). However, one study found this protocol was not safe and not efficacious to clear out infection (17), because clinical outcome of some dogs enrolled in this study was worsen at the end of study. Furthermore, long-term monitoring and exercise restriction for minimizing post-adulticide complications was problematic for application to general practice. Drug resistance from long-term use of ivermectin was also concern for this protocol. Therefore, the 2014 AHS guideline no longer recommended this protocol. However, one recent study found ivermectin combined with doxycycline could provide a safe and effective adulticidal effect, compared to melarsomine and to monthly ivermectin (4). This protocol could also reduce risk of thromboembolism and pulmonary pro-inflammatory reaction, compared to protocol using melarsomine (7). However, our study found the slow kill protocol (ivermectin and doxycycline combination) was not as efficacious as 2014 AHS protocol in order to clear out infection in dogs with HWD, although the clinical signs associated with HWD

were well resolved at the end of study (10 months after the initiation of therapy). Although recent two studies found this protocol cleared out infection in ~80% of dogs with HWD (7,9), our study found infection was cleared out in only 33% of dogs, based on antigen testing. Because current antigen test could only detect sexually intact female, the actual therapeutic efficacy of this protocol might be lower. Because prednisolone and anti-coagulant were not included in therapeutic regime in slow kill and AHS groups, the lower efficacy in this current study might be due to influence from different supportive cares.

Pulmonary hypertension (PH) is the common complication of various heart and lung diseases including degenerative valvular disease, chronic pulmonary diseases and HWD in dogs (5,6,18). Therefore, earlier and more precise evaluation of PH plays an important role in diagnosing and managing those diseases. However, diagnosis of PH in dogs generally depends on echocardiographic Doppler estimation based on tricuspid or pulmonary regurgitant jets (16), although these regurgitant jets may be undetectable or difficult to assess in many cases of dogs with PH. Several echocardiographic indices have been developed to estimate the severity of PH, including the ratio of main pulmonary artery to aorta (MPA:Ao), pulsed-wave Doppler-derived acceleration time to peak PA flow velocity (AT), and AT to the ejection time of PA flow ratio (AT:ET; 14,15). However, these indices are limited to use in clinical situation, because of relatively lower sensitivity and specificity values (e.g., MPA:Ao), and technical and alignment difficulties (e.g., AT, and AT:ET). Therefore, one recent study evaluated a right pulmonary artery distensibility index (RPADI) using 2D- and M-mode echocardiography in dogs with HWD and various degrees of PH (18). Because PH is closely related to proximal PA distension and reduced distensibility (3), RPADI has been found to be accurate and reliable diagnostic tool for estimating indirectly the severity of PH in human (2,12) and dogs (18,19). Those studies have demonstrated that the RPADI is strongly correlated to invasive PA pressures in dogs (18,19). One study suggested that RPADI value lower than 35 is indicative of PH, while 28-35 and 23-27 are indicative of mild (30-55 mmHg) and severe PH (56-79 mmHg) in dogs (18). Furthermore, RPADI value lower than 23 indicates very severe PH (> 79 mm Hg; 20). This study also suggested that the RPADI was useful to detect PH in HWD dogs without detectable TR (18). In our study population, PH was found in all dogs but 50% of dogs in both groups had detectable TR in Doppler echocardiography, although all dogs in both groups at the first presentation (D0) had lower AT/ET than reference range (i.e., < 0.42). In addition, the RPADI in all dogs of both groups was lower than reference range. Those two results strongly indicated that PH was more common complication even in dogs with mild clinical signs of HWD, although Doppler study failed to detect TR or pulmonary insufficiency. Our study also demonstrated that either slow kill or AHS protocol could resolve clinical signs associated with HWD, because the echocardiographic evidence of PH in most dogs in both groups was not detectable at the end of study (D300), although 1/6 dogs in slow kill group and 2/6 dogs in AHS group still had radiographic signs of pulmonary disease at D300. Several factors can contribute to the development of PH in dogs with HWD. Partial or complete obstruction of pulmonary blood flow into lower pulmonary vasculature by heartworm and thrombosis can be a major cause of PH in dogs with HWD. Our study clearly demonstrated the presence of PH in dogs with mild clinical signs of HWD, and thus clinician should have more attention on this complication during adulticidal therapy in dogs with milder clinical signs.

Anemia and leukocytosis with eosinophilia are the most common findings in routine hematology in dogs with HWD and can be severer with progression of disease (13). However, in this study, the anemia was noticed in only 2/6 dogs in slow kill group and leukocytosis was not noticed in dogs of both groups. It might be because the study population was only selected from Class II of HWD (mild clinical signs). In contrast, eosinophilia was noticed in most dogs (4/6 in slow kill and 6/6 in AHS group), despite mild clinical signs of HWD, suggesting that presence of eosinophilia in hematology might be useful for discriminating affected dogs with equivocal HWD antigen test. Furthermore, eosinophilia was persistently noticed in 50% of dogs in slow kill at the end of study (D300). In this group, heartworm infection was not cleared out in 4/6 dogs at the end of study, although clinical signs of all dogs were resolved. This finding suggested that persistent eosinophilia after adulticidal therapy might be indicative of therapeutic failure. Therefore monitoring on the presence of eosinophilia might be useful to discriminate unsuccessfully treated HWD dogs with equivocal result in HWD antigen test.

A limitation of this study was the low number of dogs enrolled. Moreover, right ventricle function was not studied. Right ventricle function could affect tricuspid regurgitation velocity. Furthermore, only dogs with AT > 64 ms and AT/ET ≥ 0.42 were considered normal. These cut off values have proven highly sensitive (100%) but less specific (63% and 38% respectively) in dogs (14). False positive dogs may therefore have been included in the PH subjects.

In conclusion, this study evaluated the clinical outcome of adultcidal therapy in dogs with class II stage of HWD after treating either AHS or slow kill protocol for 10 months. Clinical outcome after therapy was evaluated by clinical, radiographic and echocardiographic examination along with hematology before (D0) and after the end of therapy (D300). Our study findings suggested that the slow kill protocol might not be efficacious enough to clear out HWD in dogs and more attention on the presence of pulmonary hypertension might be necessary for effective management of HWD in dogs.

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