Response of Dogs Treated with Ivermectin or Milbemycin Starting at Various Intervals after *Dirofilaria immitis* Infection*

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**ABSTRACT**

The response to heartworm infection before preventative programs were started was investigated in 56 dogs. Dogs were infected with third-stage larvae of *Dirofilaria immitis* and started on preventative programs (monthly treatment) with ivermectin/pyrantel pamoate (IVM/PP) or milbemycin oxime (MO) 3.5, 4.5, 5.5, or 6.5 months after infection. Each time period comprised a group of six dogs treated with IVM/PP and six treated with MO. Thoracic radiographs were obtained prior to infection, at the start of preventative treatment, and at regular intervals until dogs were necropsied 1 year after the preventative was started. All dogs developed radiographic signs of heartworm disease, and all had heartworm-related arterial changes at necropsy. From Day 210 to 330, interstitial lung disease was less severe in dogs started on MO 3.5 months after infection than in dogs given IVM/PP at the same time. Arterial surfaces were more severe at necropsy in the dogs started on MO at 4.5 months than in the dogs started on IVM/PP at the same time. There was increased caudal lobar arterial and interstitial disease in the dogs treated with IVM/PP compared with dogs treated with MO; this was attributed to the death of young worms within the caudal pulmonary arteries. Dogs started on either preventative at 5.5 and 6.5 months after infection had radiographic changes and necropsy evaluations that were similar to those of untreated controls. This study reinforces the recommendation of the American Heartworm Society that mature dogs be evaluated for infection prior to starting a monthly preventative and that any dog that tests positive by a heart-
worm antigen test receive treatment with an adulticide prior to starting a heartworm preventative program.

**INTRODUCTION**

Ivermectin (IVM) and milbemycin oxime (MO) are approved for the prevention of heartworm infection by their effect on infective third- and fourth-stage larvae of *Dirofilaria immitis*. Both drugs have been shown to be highly efficacious in preventing heartworm infections when used in clinical practice. Ivermectin has been reported to be more effective than MO in killing young adult heartworms when started at 4 months after injection of infective third-stage larvae, a time when young adults have reached the pulmonary arteries; however, these reports addressed only the development of worms during this defined stage. The latest time in the heartworm life cycle when preventative treatment is effective has not been reported. Data are also lacking for dogs’ responses to heartworm infection when a preventative is initiated later. In contrast to findings of a greater impact of IVM on young adult worms, studies have demonstrated that MO had a greater impact on microfilariae concentrations.

The present study was initiated to examine the effect of MO and IVM on young heartworms and to determine whether worm death produces lung and pulmonary arterial disease. Specifically, this study sought to characterize the disease response to developing heartworm infections when heartworm preventative programs are delayed until 3.5, 4.5, 5.5, and 6.5 months after infection.

**MATERIALS AND METHODS**

**Animals and Research Design**

Fifty-six mature beagles, including equal numbers of males and females, were randomly allocated to nine groups. All dogs were injected with 100 infective third-stage larvae of *D. immitis* at the start of the study (Day 0). The dogs were then separated into eight groups of six and one group of eight. Four groups of six dogs each were randomly assigned to receive MO (Interceptor® Flavor Tabs, Novartis Animal Health, Inc., Greensboro, NC) monthly beginning 3.5, 4.5, 5.5, or 6.5 months after infection. Another four groups of six dogs were assigned to receive IVM and pyrantel pamoate (IVM/PP) (Heartgard® Plus Chewables, Meri-al Limited, Duluth, GA) at the same times as for MO. Preventatives were given according to the manufacturers’ recommendations. The ninth group of eight dogs were nontreated, infected controls.

**Radiography**

Thoracic radiographs were obtained for all dogs prior to infection and 90, 210, 240, 270, 300, 330, 360, 390, 420, 450, and 465 days after infection. Radiographs were obtained for all surviving dogs on Days 482, 493, 511, 521, 540, and 555, following necropsy of representative dogs beginning on Day 465. Radiographs were exposed with dogs placed in right lateral recumbency and in ventrodorsal position and were interpreted by a veterinarian, who was aware only of the dog’s identification number and the timing of the radiographic exposure, using a visual analog scale. Right heart size, main pulmonary arterial size, and interstitial pattern in the right caudal, left caudal, and other lobes were rated on a scale of 0 to 10, with 0 being normal, 3 being slight changes, 5 being moderate changes, 7 being severe changes, and 10 representing the most severe changes possible. Representative radiographs were used as references to reduce potential interpreter inconsistency over the time required for review of all radiographs. Alveolar pattern rating was based on the percentage of diseased lung lobe (Table 1).

The diameter of the ninth rib and caudal
pulmonary arteries on both sides was measured at their intersections using a digital caliper, which was standardized to zero before each measurement. Measurements were electronically recorded into a commercial spreadsheet, which was preprogrammed to calculate the ratio of arterial diameter divided by rib diameter.

Necropsy and Histopathology

Dogs that began their assigned preventative at 3.5 months after infection were euthanized on Day 465. Dogs started at 4.5 months were euthanized on Day 495; those started at 5.5 months were euthanized on Day 525; and the final groups started on preventatives 6.5 months after infection were euthanized on Day 555. The eight infected control dogs were euthanized on Day 555.

One hour before euthanasia, each dog was injected with 1% Evans blue dye solution in Tyrode’s buffer (pH 7.4; 2 ml/kg body weight). Dogs were euthanized with sodium pentobarbital given soon after intravenous administration of 5000 U of heparin. The heart and lungs were removed after ligation of the cranial portion of the precava and the caudal portion of the post vena cava. A cannula was placed into the precava to flush approximately 500 ml of Tyrode’s solution through the right heart and pulmonary vasculature until the effluent cleared of blood and the normal portions of lungs developed a white appearance. The lungs were inflated using an anesthetic rebreathing bag to facilitate photography with a digital camera. Photographs were taken of the dorsal and ventral surfaces of the entire lung and the caudal lung lobes. The pulmonary arteries were then dissected by the parasitologist to identify and count heartworms (data not presented). The lung, with its longitudinally opened arteries, was then photographed in its entirety.

Histologic sections were examined from 13 randomly selected dogs, including four controls, two dogs from each of the preventative groups initiated at 4.5 months, and five from groups started on preventatives at 6.5 months (three IVM/PP and two MO). Representative histopathologic samples were taken from the lung around the caudal pulmonary arteries to determine whether the heartworm disease in these dogs was typical of that previously reported as responses to live and dead worms. Digital photographs of the lungs of all dogs were examined, and dogs were scored and grouped according to the severity of arterial

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<th>TABLE 1. Rating System for Radiographic Evaluation of Alveolar Pattern Based on Percentage of Diseased Lung Lobe</th>
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<td><strong>Alveolar Pattern Rating</strong></td>
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<th>TABLE 2. Scoring of Arterial and Pleural Changes for Dogs Given 100 Infective Larvae of <em>Dirofilaria immitis</em> and Treated Monthly with Heartworm Preventatives</th>
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<td><strong>Score</strong></td>
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and pleural changes as indicated in Table 2. The heart and lungs were examined grossly and the number of heartworms determined.

Statistical Analyses

The statistician was unaware of treatment group assignments until analyses were completed. Repeated measures analysis of variance (ANOVA) methods were used to determine if differences existed among all treatment groups for all parameters. Because of significant time and/or group interactions or nonconvergent algorithms the repeated measures ANOVA was replaced by an ANOVA followed by Duncan’s multiple range test at every time point to determine if differences existed among any of the treatment groups. Means of arterial scores at necropsy and the final radiographic measurements (or evaluations) were compared between the treated groups of dogs at each time interval using Student’s *t*-test. Significance was declared when \( P < .05 \).

RESULTS

There were no differences among groups for the severity of interstitial disease or artery:rib ratios before infection or during the period prior to initiation of heartworm preventatives. All dogs except two treated with IVM/PP at 3.5 months after infection had live heartworms at necropsy (data not presented). Pulmonary arterial diameter and subjective assessment of interstitial disease in the caudal pulmonary lobes were variables that demonstrated the greatest degree of change during heartworm infection (Figures 1 and 2). Subjective assessment of right ventricular and main pulmonary arterial sizes also changed over time. All dogs had radiographic signs of heartworm disease at some time during their infection, and all had some degree of arterial disease at necropsy. Signs of alveolar disease were seldom detected and did not appear to be clinically significant in any dog.

Interstitial disease scores and artery:rib ratios in the right caudal lung lobe from groups that started preventative treatment 3.5 months after infection are presented in Figures 3 and 4. Interstitial disease was significantly less severe (\( P < .05 \)) in the group treated with MO starting at 3.5 months after infection than in either the control group or the corresponding IVM/PP group at all evaluations from 210 to 330 days after infection. Results were similar for interstitial disease signs in the left caudal lung lobe. Right caudal lobar arterial diameter ratio was significantly greater (\( P < .05 \)) in the IVM/PP group started at 3.5 months than for the corresponding MO group on Days 270 and 300 and was significantly greater (\( P < .05 \)) for the IVM/PP group started 4.5 months after infection than for the corresponding MO group on Day 330. No other measured arterial differences were detected between the two preventative treatments when they were initiated either 3.5 or 4.5 months after infection. Interstitial disease scores increased in both the right and left caudal lobes to reach the most severe scores observed (usually 5 to 6) on Days 240 to 330. Thereafter, scores of interstitial disease in the 3.5- and 4.5-month groups (for either preventative) appeared to decrease in severity; however, this score was not significantly changed for any group over time. Although some groups had lower numbers of adult worms at necropsy, indicating that worms had died during the study, alveolar disease as a reflection of worm death was seldom identified.

Interstitial disease scores and artery:rib ratios in the right caudal lung lobe from groups started on preventative treatment 5.5 months after infection are presented in Figures 5 and 6. Although radiographic changes demonstrated considerable disease, no significant differences in interstitial disease and artery:rib ratios were identified between the two groups started on preventatives at 5.5 months after infection or
tatives started at 5.5 or 6.5 months and for nontreated controls. Right ventricular and main pulmonary arterial sizes were increased over time in all groups, but differences were not consistently identified.

Mean scores of the arterial surfaces at necropsy were not significantly different between MO and IVM/PP groups started on preventative 5.5 and 6.5 months after infection, but scores for the MO group started at 4.5 months were more severe than for the matching IVM/PP group. Numbers of dogs evaluated in the groups started 3.5 months after infection were insufficient for analysis. Comparisons of mean arterial surface scores from the final radiographic evaluations just before necropsy did not reveal any significant differences between treatments for any of the four treatment-interval groups. Representative photographs from opened arterial surfaces and histologic sections are presented in Figures 7 to 14.

Histologic sections from all lungs had evidence of heartworm infection, and all dogs had larger pulmonary arteries with the villous proliferation characteristic of heartworm infection. Some villi were associated with inflammation or thrombosis. One control and one dog from the
IVM/PP group started at 4.5 months had granulomatous inflammation in the villi that was associated with pieces of dead worms. Eight of 13 dogs examined had intimal fibrosis in the smaller pulmonary arteries. In addition, 10 of the 13 dogs evaluated had changes in the parenchyma (respiratory portions of the lung); the most common change was parenchymal fibrosis, which was present in nine of the 10 affected animals.

**DISCUSSION**

Ivermectin and MO are excellent heartworm preventative when used according to label recommendations but fail as treatments in terms of blocking further development of adult worms, antigenemia, and arterial and interstitial lung disease. Although IVM/PP reduced worm survival when started 3.5 and 4.5 months after infection with a large number of infective heartworm larvae, it did not reduce pulmonary disease or antigenemia (data not presented) when started at these time intervals. Increased arterial and interstitial disease in the present study was attributed to worm death when IVM was started 3.5 or 4.5 months after infection. Administration of a heartworm preventative monthly starting 5.5 and 6.5 months after heartworm infection appeared to have no effect on severity of arterial and lung disease. Dogs started at these intervals appeared to develop disease similar to the untreated animals during the study.

Although all dogs developed arterial and in-
infection. Patients with subclinical heart disease can develop congestive failure when subjected to strenuous exercise,\textsuperscript{13} and infected dogs have increased pulmonary disease when subjected to regular exercise over a prolonged period.\textsuperscript{14} In contrast, heartworm-infected dogs with mild pulmonary hypertension tolerated a gradual introduction of a daily 20-minute treadmill exercise program at 11.3 km/hr on a 9% incline and did not develop heart failure or an increase in pulmonary hypertension.\textsuperscript{15}

All dogs in this study, including two with no worms at necropsy, developed arterial disease (interstitial) and tested positive for heartworm antigen. If any of these dogs had been evaluated as client-owned pets, the positive antigen test and radiographic evidence of lung disease would have made them candidates for treatment with melarsomine dihydrochloride (Immiticide\textsuperscript{®}, Merial Limited). Melarsomine is approved as an adulticide by the FDA, and its use is recommended according to guidelines of the American Heartworm Society.\textsuperscript{1} Treatment with melarsomine provides a known time and sequence for worm death. Appropriate use of the adulticide provides an opportunity to evaluate the severity of heartworm disease of a patient and to modify ancillary treatment, including exercise restriction. The severity of arterial and interstitial changes seen on radi-
ographs would have been sufficient criteria to use an alternate treatment regimen for melarsomine, which involves a single intramuscular injection followed by the standard two-injection sequence given at least 1 month after the initial treatment, and repeated evaluation of the patient’s disease status. This approach spreads the effect of worm kill over two episodes of treatment, as opposed to using a preventative after infection has already developed.

Arterial and interstitial disease in the right caudal lung lobe appeared to be worse in the IVM/PP group that started treatment at 3.5 months than in the corresponding MO group. If this is true, then decreased worm development when IVM/PP is started at 3.5 months after infection may actually increase arterial disease associated with worm death. Trickle kill apparently produces arterial disease, which may partially resolve during the first year after starting the preventative. The sequence of pulmonary arterial and lung disease in response to live worms is well documented. The initial arterial response includes endothelial swelling, widened intercellular junctions, sloughing of longitu-

Figure 3. Interstitial disease scores for the right caudal lung lobe in heartworm-infected dogs started on monthly heartworm preventatives 3.5 months after infection. Asterisks indicate that the mean score for dogs treated with milbemycin oxime is significantly less (P < .05) than that for nontreated controls and dogs treated with ivermectin/pyrantel pamoate.

Figure 4. Ratios of right caudal pulmonary artery:rib diameter at their intersection at the ninth rib as viewed on ventrodorsal radiographs of dogs started on monthly heartworm preventatives 3.5 months after infection. Asterisks indicate that the mean score for dogs treated with milbemycin oxime is significantly less (P < .05) than that for nontreated controls and dogs treated with ivermectin/pyrantel pamoate.
to arterial flow producing pulmonary arterial hypertension and thickened, dilated arterial walls. These arterial changes can be seen with survey radiographs within a few weeks of worms entering the arteries and were demonstrated throughout the current study and accompanied by the classical gross and histologic appearance of heartworm disease evident at necropsy. In contrast, dying and dead worms are swept distally into the arteries to produce an accelerated arterial surface injury, thromboembolism, and inflammation. In addition to worsening of arterial disease, the increased inflammation and permeability produces alveolar disease around the diseased arteries. These changes can be seen radiographically and may progress to clinical signs of severe coughing, dyspnea, and even hemoptysis. In the current study, many dogs, including two without any live worms, had pulmonary changes typical of responses to dead worms. The severity of these disease responses can be partially reduced and modified by medical treatment.

To recognize when changes associated with worm death are occurring in a client-owned dog, it is important that the timing of drug treatment be known and that the patient be closely moni-
Figure 7. Opened arterial view (A) and microphotograph (B) of lung from a heartworm-infected control dog. Arterial changes were severe (score = 4). There were 25 male and 21 female worms present. Typical lesions included severe villous proliferations from the intima and a large thrombus.

Figure 8. Opened arterial view (A) and photomicrograph (B) of lung from a heartworm-infected control dog. Arterial changes were severe (score = 4). There were 19 male and 23 female worms present. Typical lesions included multiple villous proliferations from the intima.
Figure 9. Opened arterial view (A) and photomicrograph (B) of lung from a heartworm-infected dog started on ivermectin/pyrantel pamoate 6.5 months after infection. Arterial changes were severe (score = 4). There were 16 male and 16 female worms present. Lesions included extensive villous proliferations.

Figure 10. Opened arterial view (A) and photomicrograph (B) of lung from a heartworm-infected dog started on milbemycin oxime 6.5 months after infection. Arterial changes were severe (score = 4). There were 26 male and 12 female worms present. Lesions included very severe arterial intimal fibrosis that is almost occluding the lumen of the distal caudal lobar artery.
Figure 11. Opened arterial view (A) and photomicrograph (B) of lung from a heartworm-infected dog started on ivermectin/pyrantel pamoate 4.5 months after infection. Arterial changes were severe (score = 2). One male and four female worms were recovered. Medial hypertrophy and villous proliferations were present, including serum-filled cysts surrounded by mineralization, multinucleated cells, and fibrosis, which are probably the remnants of granulomatous inflammation around dead worms.

Figure 12. Opened arterial view (A) and photomicrograph (B) of lung from a heartworm-infected dog started on milbemycin oxime 4.5 months after infection. Arterial changes were severe (score = 2). There were 23 male and 19 female worms present. Lesions included mild villous proliferations of the intimal surface of a larger caudal pulmonary arteries.
Figure 13. Collage of opened arterial views from four heartworm-infected dogs started on ivermectin/pyrantel pamoate 6.5 months after infection and necropsied 12 months after initiation of treatment. The heartworm mass was typical of the worm burdens (group mean = 30.3) recovered from dogs in this group.

Figure 14. Collage of opened arterial views from four heartworm-infected dogs started on milbemycin oxime 6.5 months after infection and necropsied 12 months after initiation of treatment. The heartworm mass was typical of the worm burdens (group mean = 38.7) recovered from dogs in this group.
tored. Exercise must be restricted, and other treatments may be indicated following adulticide treatment.

This study confirms the importance of preventing heartworm infection and reinforces the recommendation to test dogs 6 to 12 months after starting a preventative. The effect of IVM on young adult worms has been related to a decreasing antigen concentration. Since currently available antigen tests are very sensitive, these low antigen concentrations are still detected and are indicative of worm infection. The impact of macrolides on decreasing microfilariae concentrations and eventual production of a microfilariae-negative condition (occult adult infections) and changes in the embryograms of female worms in treated dogs further reinforce the recommendation to use antigen tests for screening. Microfilarial concentration tests during heartworm prophylaxis are insensitive detectors of infections.

REFERENCES


