Focus on Canine Heartworm Disease

Molly D. Savadellis, Research Professional II, BS, PhD Candidate
University of Georgia

Heartworm disease (HWD) is caused by the filarial nematode *Dirofilaria immitis*. This parasite has a necessary mosquito intermediate host and has been found in many different mammalian species, including cats, dogs, monkeys, marine mammals, and rodents. Wild and domestic canids are the most prevalent hosts infected with *D. immitis* and have the largest adult worm burden.

Mature, sexually reproductive adults are located in the right heart chambers and the pulmonary and lobar arteries. These adults produce microfilariae in the circulating bloodstream, available to be ingested by female mosquitoes during a blood meal.

The ingested microfilariae develop into the infective third-stage larvae (L3) after several molts. These L3 migrate to the mosquito head and mouthparts where they are deposited in hemolymph onto mammalian skin during subsequent blood meals. After the deposited L3 enter the host through the mosquito bite wound, they molt to fourth-stage larvae (L4). The L4 then migrate through the tissues toward the heart.

Usually by day 90 to 120 post-infection, all of the worms have reached the main pulmonary artery and are mature adults.

All *D. immitis* parasite life stages contain the endosymbiont bacteria *Wolbachia*, which is necessary for successful larval development and sexual reproduction. The American Heartworm Society (AHS) recommends treating heartworm-positive dogs with doxycycline to reduce the concentration of *Wolbachia* present during adulticide treatment, thereby reducing the inflammatory immune response to the bacteria.
Heartworm preventives target L3 and L4 using macrocyclic lactones (MLs) such as ivermectin, selamectin, milbemycin oxime, and moxidectin. Recently, suspected ML-resistant *D. immitis* isolates have been identified. With the emergence of potential ML resistance, additional drugs capable of targeting heartworms and preventing HWD and a better understanding of our current HWD treatments are necessary.

**Overview of Selected Literature**

**Carretón and colleagues** examined cardiopulmonary biomarkers and renal parameters during canine adult heartworm treatment. The classic 2-injection melarsomine treatment and the AHS’s recommended 3-injection melarsomine treatment—with and without prednisone—were compared. The dogs receiving the AHS-recommended heartworm treatment protocol experienced fewer and less severe clinicopathologic abnormalities compared with those receiving the 2-injection protocol.

**McCall and colleagues** evaluated the effect of doxycycline on canine heartworm microfilariae and adult worms. Doxycycline was administered at 10 mg/kg PO q12h for 30 days as recommended by the AHS during canine adulticide treatment. A reduction in concentration of microfilariae post-doxycycline treatment was observed. Additionally, doxycycline treatment appeared to alter the morphology and motility of live adult heartworms recovered approximately 1 year post-treatment. Doxycycline-treated microfilariae fed to mosquitoes developed into infective L3. These L3 were injected into naïve dogs. At both 7 and 10 months post-infection of all naïve dogs, no live or dead adult heartworms were recovered. The results of this study suggest that doxycycline may be a successful adjunctive treatment against the transmission of potential ML-resistant heartworms.

**Chandrashekar and colleagues** evaluated the efficacy of doxycycline and Advantage Multi (bayerdvm.com) on immature adult *D. immitis*. Doxycycline was administered at 10 mg/kg PO q12h for 30 days, and imidacloprid 10 mg/kg + moxidectin 2.5 mg/kg was applied topically every 30 days. This treatment regimen was 100% effective against approximately 3.5- and 5-month-old immature adult heartworms in preventing the development of canine HWD. This treatment regimen can be used to prevent HWD in dogs that have not been administered heartworm prevention for 2 to 5 months.

**Bourguinat and colleagues** identified and confirmed 2 separate *D. immitis* isolates of ML resistance. Using populations of ML-susceptible and ML-resistant worms, whole genome analysis identified 6 loci containing the greatest genetic variation. These loci may be used in developing a genetic test to differentiate between ML-susceptible and ML-resistant isolates.

Veterinarians must keep up with the guidelines for treating canine HWD, as well as determine if a case is potentially resistant to MLs. The articles below will facilitate heartworm treatment and management decisions made by veterinarians.

**Evaluation of cardiopulmonary biomarkers during classic adulticide treatment versus the American Heartworm Society recommended treatment protocol in dogs infected by *Dirofilaria immitis***


During adulticide treatment of dogs with HWD, the death of adult worms may cause pulmonary thromboembolism, pulmonary inflammation, congestive heart failure, or renal disease. Cardiopulmonary biomarkers and renal parameters can be used to monitor these potential adulticide side effects.

The aim of this study was to evaluate biomarkers during the classic 2-injection protocol and the AHS-recommended heartworm treatment protocol by measuring cardiac troponin I (cTnI), myoglobin, D-dimers, blood urea nitrogen (BUN), creatinine, and urine protein:creatinine (UPC).

The biomarker cTnI is released during myocardial inflammation, ischemia, or necrosis. D-dimers have been used to evaluate the presence of microthrombi and thromboembolism. Renal function can be measured by BUN, creatinine, and UPC concentrations.

Fourteen dogs with confirmed *D. immitis* antigen and microfilariae were used in this study. Dogs were randomly assigned to a treatment protocol group.

Group 1 dogs (n=5) received 2 melarsomine injections 24 hours apart. Dogs in group 2 (n=5)
and group 3 (n=4) received monthly ivermectin at 6 mcg/kg and 4 weeks of doxycycline at 10 mg/kg PO q12h after diagnosis. Thereafter, dogs received melarsomine injections at 2.5 mg/kg on days 60, 90, and 91. Dogs in group 3 also received prednisone, per AHS guidelines, after the first and third melarsomine injection on days 60 and 91 (Table).

Blood and urine samples were obtained on days 0, 7, and 14 after the first melarsomine injection, and 30 days after the last melarsomine injection.

**STUDY RESULTS**

- Elevations of **D-dimers** were mild to moderate.
- A higher prevalence of dogs presented with elevated **D-dimers** after receiving the classic treatment, although this was not statistically significant.
- The highest mean **D-dimer** concentrations occurred in dogs receiving the classic treatment and in dogs receiving prednisone.
- 30 days after the last melarsomine injection, only 20% of dogs in groups 2 and 3 had pathologic concentrations of **D-dimer**, whereas 60% of dogs in group 1 presented with mild to moderate elevations of D-dimers.
- **cTnI** levels were mildly elevated and decreased to normal levels in all study dogs post-adulticide treatment.
- **Blood myoglobin** levels were mildly elevated in group 1 dogs throughout treatment.
- **BUN** and **creatinine** concentrations were within normal ranges for all dogs throughout treatment.
- Group 1 dogs’ **UPC** values did not significantly change post-treatment; mean values for UPC were less than the cutoff value for clinical diagnosis of proteinuria in all groups at all time points.

**CONCLUSIONS**

In analyzing the biomarkers used throughout this study, fewer and less severe abnormalities were documented in the dogs receiving the AHS-recommended heartworm treatment protocol.

Although a higher prevalence of dogs presented with elevated D-dimers after receiving the classic treatment (group 1) compared with groups 2 and 3, this was not statistically significant and must be interpreted in light of the small numbers of dogs in this study, and in respect to the timing of the final blood sample. This 30-day post-melarsomine blood sample was obtained on day 121 for groups 2 and 3, while group 1 was collected on day 32.

These findings indicate that the current AHS-recommended canine HWD treatment may produce less severe side effects (ie, thromboembolism) compared with the classic adulticide treatment. Therefore, this study supports the use of the AHS-recommended adulticide treatment.

**TABLE** Heartworm Adulticide Treatment Protocols

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NUMBER OF DOGS</th>
<th>DRUG PROTOCOL</th>
<th>FINAL BLOOD AND URINE SAMPLE COLLECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5</td>
<td>Melarsomine 2.5 mg/kg IM q24h for 2 doses&lt;br&gt;• Ivermectin 6 mcg/kg every month&lt;br&gt;• Doxycycline 10 mg/kg PO q12h for 4 weeks&lt;br&gt;• Melarsomine 2.5 mg/kg IM on days 60, 90, and 91</td>
<td>Day 32</td>
</tr>
<tr>
<td>Group 2</td>
<td>5</td>
<td>Melarsomine 2.5 mg/kg IM q24h for 2 doses&lt;br&gt;• Ivermectin 6 mcg/kg every month&lt;br&gt;• Doxycycline 10 mg/kg PO q12h for 4 weeks&lt;br&gt;• Melarsomine 2.5 mg/kg IM on days 60, 90, and 91</td>
<td>Day 121</td>
</tr>
<tr>
<td>Group 3</td>
<td>4</td>
<td>Melarsomine 2.5 mg/kg IM q24h for 2 doses&lt;br&gt;• Ivermectin 6 mcg/kg every month&lt;br&gt;• Doxycycline 10 mg/kg PO q12h for 4 weeks&lt;br&gt;• Melarsomine 2.5 mg/kg IM on days 60, 90, and 91&lt;br&gt;• Prednisone PO on days 60 and 91—0.5 mg/kg BID for the first week, SID for the second week, and every other day for 2 weeks</td>
<td>Day 121</td>
</tr>
</tbody>
</table>
Effects of doxycycline on heartworm embryogenesis, transmission, circulating microfilaria, and adult worms in microfilaremic dogs


With the emergence of ML resistance, there is a need for additional drugs capable of targeting multiple D. immitis life stages. The tetracycline doxycycline is prescribed during canine heartworm adulticidal treatment to reduce the concentration of the endosymbiont bacteria Wolbachia and the pathologic inflammatory response caused by the death of adult worms and subsequent release of Wolbachia.

Doxycycline has been demonstrated to prevent embryogenesis and development of larval stages and even to slowly kill adults in several filarial species harboring Wolbachia.

The aim of this study was to evaluate the development and infectivity of D. immitis microfilariae post-doxycycline treatment. The concentration of circulating microfilariae, antigenemia, and adult worm recovery were analyzed in this study.

Five heartworm-positive, microfilaremic dogs were administered doxycycline 10 mg/kg PO q12h for 30 days as recommended by the AHS before adulticide heartworm treatment. The dogs did not receive treatment with melarsomine and were housed in a mosquito-proof area; therefore, they were protected from new exposure to D. immitis. Blood samples were obtained monthly for approximately 1 year to monitor heartworm antigen status and the concentration of microfilariae.

Approximately every 2 to 3 months, mosquitoes were allowed to feed, ingesting blood with microfilariae that had the potential to develop into infective L3. The L3 were then injected into naïve dogs to monitor infectivity post-doxycycline treatment. Doxycycline-treated dogs were necropsied approximately 1 year post-treatment, and overall adult worm recovery and morphology were analyzed.

STUDY RESULTS

- **Circulating microfilariae concentration** in doxycycline-treated dogs dropped below the level of detection after approximately 164 days post-treatment.
- **Heartworm antigen** was detected throughout the study in both control and doxycycline-treated dogs.
- **Dead and live adult worms** were recovered from doxycycline-treated dogs.
- **Live adult worms** recovered from doxycycline-treated dogs were abnormal in appearance and motility.
- **Live adult worms** recovered from control dogs were normal in motility and appearance.
- **No heartworm antigen nor detectable circulating microfilariae** were present in naïve dogs receiving L3 developed from microfilariae collected from dogs approximately 2.5 months after doxycycline therapy; no live or dead worms were recovered from these previously naïve dogs 10 months post-infection.
- **No heartworm antigen nor detectable circulating microfilariae** were present in naïve dogs receiving L3 developed from microfilariae collected from doxycycline-treated dogs approximately 5 months post-treatment; no live or dead worms were recovered from these previously naïve dogs 7 months post-infection.

CONCLUSIONS

The AHS’s recommended adulticide treatment for canine HWD uses the tetracycline doxycycline to reduce the concentration of Wolbachia present in all parasitic life stages. Successful sexual reproduction and development of parasitic larval stages are dependent on the presence of Wolbachia.

This study demonstrated a reduction in concentration of circulating microfilariae post-doxycycline treatment, which could be attributed to an impairment of sexual reproduction of adult heartworms. The doxycycline therapy appeared to alter...
the morphology and motility of live adult heartworms recovered from infected dogs approximately 1 year after treatment.

Doxycycline-treated microfilariae fed to mosquitoes developed infective L3, which were injected into naïve dogs. At both 7 and 10 months post-infection of all naïve dogs, no live or dead adult heartworms where recovered. This indicates L3 derived from doxycycline-treated microfilariae lack the ability to successfully develop into adult heartworms.

The dogs in this controlled study were kept in mosquito-proof housing, preventing coinfection with heartworms that had not previously been exposed to doxycycline. Despite the small number of dogs in each experimental group, the data collected is compelling that *Wolbachia* does indeed play an important role in the survival, development, and infectivity of heartworms.

The results of this study suggest that doxycycline may be a successful adjunctive treatment against the propagation of potential ML-resistant heartworms. If a canine with ML-resistant HWD is treated using the AHS’s recommended treatment protocol, the use of doxycycline may prevent further transmission of resistant heartworms.

**Experimental *Dirofilaria immitis* infection in dogs:** Effects of doxycycline and Advantage Multi® administration on immature adult parasites


The MLs ivermectin, milbemycin oxime, moxidectin, and selamectin are used in the prevention of HWD by killing *D. immitis* L3 and L4, preventing the development of adult worms in the pulmonary artery. Many of these MLs have been tested for adulticidal effects.

The aim of this study was to evaluate the efficacy of doxycycline and 10% imidacloprid + 2.5% moxidectin against immature adult heartworms in experimentally infected dogs. This study specifically investigated the effect of this treatment on the concentration of microfilariae circulating in the bloodstream, antigenemia, and adult worm recovery at necropsy.

Twelve dogs were randomly assigned to 3 groups. Two dogs in each group were infected with 6 *D. immitis* L3, and 2 dogs from each group were infected with 12 *D. immitis* L3. These low numbers of L3 more closely resemble a natural infection occurring over a period of time.

The treatment regimen of 30 days of doxycycline 10 mg/kg PO q12h and imidacloprid 10 mg/kg + moxidectin 2.5 mg/kg topically every 30 days was initiated at study day 105 (3.5 months post-infection) for group A dogs and at study day 149 (5 months post-infection) for group B dogs.

Necropsy for groups A, B, and C (untreated control) were performed on study day 407 (14 months post-infection).

Blood and serum samples were collected every 2 to 4 weeks for quantification of microfilariae and detection of *D. immitis* antigen by the commercially available test kit PetChek PF HW Antigen Test ([idexx.com](http://idexx.com)).

**STUDY RESULTS**

- **No live nor dead adult worms** were recovered from groups A and B.
- **Live adult worms** were recovered from group C.
- **Microfilariae** were not detected in any experimental group throughout the course of this study.

Intermittent antigenemia was detected in groups A and B, but detectable antigenemia was not found in any treated dog by the end of the study.

Group C dogs infected with 6 L3 did not have detectable antigenemia; however, those infected with 12 L3 had detectable antigenemia that persisted until the end of the study.
CONCLUSIONS

The treatment regimen of doxycycline 10 mg/kg PO q12h for 30 days and monthly topical administration of 10% imidacloprid + 2.5% moxidectin was 100% effective against immature adult heartworms in preventing canine HWD. While this study had 100% prevention of canine HWD development, there were small numbers of dogs in each experimental group, preventing any statistical analysis of these results.

Immature adult worms would have been present primarily in the lobar arteries of these dogs when treatment was initiated. No live or dead worms were recovered at necropsy of both treated groups. This may indicate that the treatment regimen successfully killed the parasites before they developed into sexually mature adults.

In a study to be published in the near future, monthly topical administration of 10% imidacloprid + 2.5% moxidectin in combination with doxycycline 10 mg/kg PO q12h for 30 days has been evaluated against sexually mature adult heartworms. This treatment regimen resulted in the elimination of circulating microfilariae within 21 days post-treatment, and with continued topical therapy had a 95.9% efficacy in eliminating sexually mature adult heartworms after 10 months.¹

This treatment regimen can be used in veterinary practices in endemic and nonendemic areas for 2 different scenarios:

• 2- to 5-month-old dogs
• ≥6-month-old dogs that have missed 2 to 5 months of heartworm prevention

Utilizing this treatment regimen against immature adult heartworms prevents the development of sexually mature adults, greatly reducing pathology associated with HWD, and eliminating transmission of microfilariae to other animals in the area. In addition, this treatment can be prescribed without having to wait 6 to 12 months after a period of noncompliance with heartworm preventives for adult heartworms to develop and become detectable by commercially available heartworm antigen tests.

Molly D. Savadelis
Molly D. Savadelis is a PhD student at the University of Georgia in the Department of Infectious Diseases. Her dissertation work is focused on canine heartworm disease treatments utilizing the combination of macrocyclic lactones and tetracyclines and how these various treatments may affect heartworm diagnostics. She hopes to continue her career working on both canine and feline heartworm disease.
Macrocyclic lactone resistance in *Dirofilaria immitis*: Failure of heartworm preventives and investigation of genetic markers for resistance


Lack of efficacy (LOE) claims for ML heartworm preventive products have increased in locations around the United States. Many of these cases can be attributed to improper administration of these products or inconsistent compliance. Despite this, some LOE claims are suspected ML resistance.

Resistance to the ML ivermectin has recently been confirmed. Genetic markers that discriminate between susceptible and ML-resistant heartworm isolates have not been identified but may greatly facilitate testing to differentiate LOE claims. A genetic test based on molecular markers for ML-resistant phenotypes would facilitate identification and geographic mapping of ML-resistant cases, as well as guide future research to produce effective alternative drugs.

The aims of this study were to identify and confirm ML-resistant LOE cases, and then use the confirmed ML-resistant strains to search for loci within the genome associated with the resistant phenotype.

Two LOE suspected ML-resistant cases, Td2008 from Louisiana and Jd2009 from Arkansas, were used in this study. Ten beagles were infected with Td2008 strain, and 12 beagles were infected with Jd2009 strain heartworm. Thirty days post-infection with Td2008 or Jd2009, the beagles were treated with commercially available chewable ivermectin tablets administered at 0.006 to 0.013 mg/kg PO for both strains. Necropsies were then performed to recover adult heartworms.

Adult heartworm DNA from ML-susceptible populations and microfilarial DNA from ML-resistant populations were pooled for whole genome analysis. The susceptible population comprised DNA samples from Missouri strain heartworm infected dogs (TRS Labs Inc, Athens, GA) and naturally infected dogs from Grand Canary Island, Spain, Grenada, and the Po Basin in Northern Italy.

**STUDY RESULTS**

- **Efficacy of ivermectin** against the Td2008 strain was 23.8% after 9 months of treatment.
- **Efficacy of ivermectin** against the Jd2009 strain was 71.3% after 5 months of treatment.
- 186 loci were identified as potential molecular markers for ML resistance; 158 of these loci were statistically significantly different between susceptible and resistant populations.
- Six loci were identified as the best potential genetic markers for differentiating between susceptible and resistant populations, because they contained the lowest frequency of the susceptible genotype in the LOE or resistant populations.

**CONCLUSIONS**

This study confirms that the Louisiana Td2008 and Arkansas Jd2009 canine heartworm strains are indeed resistant to MLs. This was demonstrated by the development of adult heartworms despite 9 and 5 months of treatment with chewable ivermectin administered at 0.006 to 0.013 mg/kg PO for both strains.

This study observed 6 specific loci within the genome of LOE and ML-resistant populations with the lowest frequency in susceptible genotypes. The 6 loci identified contain single nucleotide polymorphisms, representing genetic variation between susceptible populations and ML-resistant and LOE populations.

While DNA was analyzed from several geographic locations for susceptible populations, these 6 loci were identified from polymorphic loci that showed no significant nucleotide difference attributable to geography between all the susceptible populations.
The 6 loci identified in this study may be used to differentiate between ML-susceptible and ML-resistant populations, but they do not identify the exact mechanism of ML resistance. Despite this, the possibility of genetic testing capable of identifying ML-resistant heartworm isolates would allow geographic mapping and surveillance to help scientists better understand the spread of ML resistance.

With this demonstrated ML resistance, it is necessary to develop a test capable of distinguishing between noncompliance and ML-resistant cases. The genetic markers characterized in this study may provide the starting point for the development of such testing. With this, LOE and suspected ML-resistant *D. immitis* cases should continue to be investigated and examined for additional genetic markers for ML resistance.

**References**
