Investigating veterinary management choices for canine heartworm disease
(*Dirofilaria immitis*) in northern Mississippi

by

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A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

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ABSTRACT

TOBI KU: Investigating veterinary management choices for canine heartworm disease (Dirofilaria immitis) in northern Mississippi (Under the direction of Dr. Clarke Atkins)

Canine heartworm disease affects nearly 45% of dogs in endemic regions of the United States (Atkins, 2005). There are concerns that the chronic use of macrocyclic lactone (ML) preventives to kill adult heartworms (“soft-” or “slow-kill”) may have contributed to the development of ML resistance. My study of this problem had three objectives: (a) to determine the frequency of “slow-kill” treatment in heartworm-positive patients and compare them to practitioner estimates; (b) to survey practitioner opinions on the factors influencing heartworm disease management; and (c) to analyze the heartworm prevention history of heartworm-positive patients in order to understand the causes of heartworm infection in this region. The study group consisted of dogs determined to be heartworm-positive when presented to a mixed-animal practice in northern Mississippi. Client records were scrutinized for heartworm preventive purchase history. Veterinarians in the four-doctor practice completed a questionnaire concerning their beliefs and practices in regard to heartworm treatment. 75% of heartworm-positive patients received “slow-kill” treatment, more than 20% greater than that estimated by the practitioners. 12.5% of patients received adulticidal treatment, equivalent to those that received no treatment. Injectable moxidectin was the most common ML preventive used in “slow-kill” treatment. Client financial concerns were cited as the primary reason for choosing “slow-
“slow-kill” treatment. The results of this study show that practitioner estimates of “slow-kill” prevalence within their clinics may be suspect in their accuracy. Despite the recommendations of the American Heartworm Society, clients and veterinarians prefer the “slow-kill” method of heartworm treatment. However, trends in patient heartworm preventive history show that poor client compliance remains the predominant reason for heartworm infection. Thus, consistent use of existing, effective heartworm preventives should be the primary goal in reducing prevalence of heartworm infection, regardless of the recognized threat of resistance. Further study is needed on the risks and efficacy of “slow-kill” treatment and the effects of different ML preventives for the treatment of heartworm infection.
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<th>Description</th>
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<tbody>
<tr>
<td>ML</td>
<td>macrocyclic lactone</td>
</tr>
<tr>
<td>HW</td>
<td>heartworm</td>
</tr>
<tr>
<td>HWD</td>
<td>heartworm disease</td>
</tr>
<tr>
<td>HWI</td>
<td>heartworm infection</td>
</tr>
<tr>
<td>HWP</td>
<td>heartworm preventive</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>LOE</td>
<td>lack of efficacy</td>
</tr>
<tr>
<td>AHS</td>
<td>American Heartworm Society</td>
</tr>
<tr>
<td>WOI</td>
<td>window of infection</td>
</tr>
</tbody>
</table>
CHAPTER 1
INTRODUCTION

Canine heartworm disease significantly impacts the United States, infecting nearly 45% of dogs in endemic regions (Atkins, 2005). Mosquitos transmit the third larval stage from heartworm-infected canines to other canine hosts. Current macrocyclic lactone heartworm preventive treatments are extremely effective at stopping canine heartworm infection by killing these larval stages. These preventives demonstrated perfect efficacy when initially approved by the Food and Drug Administration (Bowman, 2012). However, numerous lack-of-efficacy claims filed against some heartworm preventives in the last decade combined with the isolation of preventive-resistant heartworm strains have sparked increasing concern regarding the development of heartworm resistance to macrocyclic lactones (Bowman, 2012). These concerns are especially relevant in the southeast United States, which has the nation’s highest incidences of heartworm infection (Wang et al., 2014; Bowman et al., 2009) (Figure 1). In order to effectively combat canine heartworm disease, it is critical to investigate and understand how it is managed by veterinary practitioners and clients in this region.

While a small body of research on heartworm management in the Mississippi (MS) delta region exists, many of these studies rely on retroactive self-report of clinical data (Colby et al., 2014; Pulaski et al., 2016). Self-reporting can be unreliable due to
individual bias and imperfect recordkeeping. In this study, I analyzed the management of canine heartworm disease in a northern MS private practice.

Figure 1. Geographical prevalence of canine heartworm disease in the United States. Source: Companion Animal Parasite Council Parasite Prevalence Maps. Data provided by IDEXX Laboratories and ANTECH Diagnostics. Copyright ©CAPC 2017, used with permission.
Theoretical Framework

Heartworm disease (HWD) is the pathology caused by prolonged heartworm infection (HWI). The parasitic roundworm *Dirofilaria immitis* resides in the pulmonary artery and may cause vascular and respiratory damage to the canine host (Atkins, 2005). *D. immitis* has a multi-stage life cycle: the microfilarial larval stage (L1) is ingested from an infected canine host by a feeding female mosquito. After several molts inside the mosquito host, the infective third-stage larvae (L3) can be transmitted to new canine hosts through additional mosquito feeding events (Atkins, 2005). A summary of the life cycle of *D. immitis* is shown in Figure 2.

*Figure 2. Life cycle of *Dirofilaria immitis*. Images courtesy of Wikimedia Commons*
Macrocyclic lactone (ML) preventive therapy, currently the most prevalent method of HWD prophylaxis, prevents onset of HWD by killing both the deposited L3 and the next, fourth-stage larvae (L4) (Bowman, 2012). Prior to the development of ML preventives in the 1980s, daily administration of oral diethylcarbamazine was the only method of *D. immitis* prophylaxis (Kume et al., 1962). Diethylcarbamazine products fail to prevent HWI if a single day of administration is skipped, and can also cause death in dogs with preexisting HWI due to the cardiovascular blockage resulting from sudden microfilarial death (Kurokawa et al., 1963). In contrast, ML preventives are currently recommended by the AHS (2014) because of their safety, long-lasting efficacy, and ease of administration.

The two ML subgroups are the avermectins (including ivermectin and selamectin) and the milbemycins (moxidectin and milbemycin oxime). They are structurally similar to the antibacterial macrolides (McKellar & Benchaoui, 1996). While their modes of action have not been fully characterized, MLs primarily act on the glutamate-gated chloride channels of filarial excretory and secretory pores, compromising parasite ability to avoid host immune defenses (Moreno et al., 2010; AHS, 2014; Mani et al., 2016). ML preventives can be administered orally, topically, or subcutaneously and are available in many different formulations (Figure 3). These formulations may contain the ML alone or include flea, tick, and other endoparasite preventive chemicals. Cost comparisons between different HWP methods are difficult to determine accurately as prices are influenced by many factors, including veterinary practice pricing mark-ups, method of administration, broad-spectrum or heartworm-only preventives, and trade-name or generic formulations. Analyses of online distributors can provide a ballpark estimate for
the costs of heartworm prevention. For a six-month supply of heartworm prevention suitable for a 40-lbs dog, oral heartworm-only preventives such as milbemycin oxime (Interceptor®, Elanco Animal Health, Greenfield, IN) typically cost around $40.00, while oral combination preventives containing milbemycin oxime and the flea preventive spinosad (Trifexis®, Elanco Animal Health) cost around $110.00 (PetMed Express, 2017). According to the manufacturer, the injectable heartworm-only preventive containing moxidectin costs $45.96 on average for a six-month dose (Lavan & Login, 2015).

Figure 3. Administration of macrocyclic lactone heartworm preventive products.
Resistance to macrocyclic lactones. Although ML preventives met perfect standards of efficacy when they were first introduced, there is increasing evidence that *D. immitis* may be developing resistance to these compounds. The Food and Drug Administration (FDA) Center for Veterinary Medicine saw a dramatic increase in lack-of-efficacy (LOE) claims in the beginning of the 21st century, especially in HW-endemic areas of the southeast United States (Hampshire, 2005). Clinical practices typically file LOE claims to pharmaceutical companies that offer compensation for cases of HW infection in which documentation of consistent HW prevention has been maintained, usually in the form of client purchase history, and the FDA Center for Veterinary Medicine further compiles and analyzes these LOE submissions. Additionally, a 2014 survey found that 74% of all Louisiana veterinary practices had seen at least one LOE case within the last year (Pulaski et al., 2014), and preliminary results from currently unpublished, ongoing questionnaires throughout the MS delta region (Mississippi, Missouri, Alabama, Arkansas, and Louisiana) seem to follow this trend (Pulaski et al., 2016). Other researchers have discovered and isolated *D. immitis* microfilaria (L1) that persist through ML treatment; for example, during the development of new ML preventive combinations, it was found that previously effective compounds were no longer demonstrating perfect efficacy with certain *D. immitis* strains (Bowman, 2012). In recent years, multiple research teams have successfully isolated ML-resistant *D. immitis* strains from LOE cases (Bowman, 2012; Pulaski et al., 2014). Thus, it is well accepted that some heartworm isolates are resistant to the effects of ML preventives.

Despite these findings, the impact of *D. immitis* resistance is poorly understood, in part due to the complex nature of the parasite’s life cycle. In suspect resistant *D. immitis*
isolates, certain larval stages are more susceptible to the effects of ML preventives than others, such as microfilaria (L1) (Bowman, 2012). Certain therapeutic practices, such as the use of ML as a “slow-kill” adulticide in treating heartworm infection (HWI), have been suggested as contributing to resistance because they can target highly susceptible larval stages while allowing more resistant stages to multiply (Bowman, 2012). In other words, such practices may allow for selection of resistance in *D. immitis*. Additionally, although ML resistance is often the suspected cause of ML treatment failures in practice, most LOE cases are likely caused by poor compliance to HW preventives leading to inconsistent protection coverage (Boman, 2012; Atkins et al., 2014).

**American Heartworm Society recommendations for heartworm infection treatment.** The American Heartworm Society (AHS), an international organization founded in 1974, maintains guidelines for the prevention, diagnosis and treatment of HWD. The AHS hosts the triennial American Heartworm Symposium, and in 2013 the symposium addressed the increasing prevalence of LOEs and ML-resistant isolates. These discussions informed the development of the AHS’s *Current Guidelines for the Prevention, Diagnosis, and Management of Heartworm (Dirofilaria immitis) Infection in Dogs* (2014). According to these guidelines, the AHS recognizes melarsomine dihydrochloride (Immiticide®, Merial Limited, Duluth, GA) as the only FDA-approved adulticidal therapy for HWI. Melarsomine dihydrochloride is an arsenical molecule that causes death in heartworms that are over four months old, and it is effective for the treatment of canine heartworm infection (McTier et al., 1994). The AHS also recommends the use of doxycycline in combination with melarsomine adulticidal
protocols. Doxycycline targets *Wolbachia*, endo-symbiotic bacteria that reside in *Dirofilaria immitis* at all life stages and have been implicated in HWD pathogenesis, as *Wolbachia* surface protein has been shown to induce host antibody and inflammatory responses (Kramer et al., 2005). Thus, doxycycline therapy has been shown to reduce the chance of adverse complications and mortality over the course of HW treatment (AHS, 2014).

*“Slow-kill” therapy.* While regular ML preventive administration is included in the AHS’s recommended HW treatment protocol, the continuous use of ML preventives in a HW-positive canine *without melarsomine* with the intent of adulticidal action is known as “slow-kill” therapy (AHS, 2014). This is considered an off-label use of ML preventive products because they are not approved by the FDA for treating HWI; the mechanism of action for “slow-kill” therapy is yet to be understood. “Slow-kill” therapy first became prevalent in 2010, when melarsomine began to fall into short supply due to manufacturer delays (Becker, 2011); according to the FDA, the melarsomine shortage is still unresolved (U.S. Food and Drug Administration, 2016). While studies have demonstrated the adulticidal effect of several ML preventives especially in combination with doxycycline (McCall et al., 1996; Venco et al., 2004; Bazzocchi et al., 2008; Chandrashekar, 2014), the AHS (2014) asserts that the delayed adulticidal effects of these products allow unsafe HWD pathology to continue to progress as the worms die slowly. Additionally, as previously discussed, there is a possibility that the use of ML preventives in “slow-kill” therapy exacerbates the evolution of HW resistance to ML (Bowman, 2012). For these and other reasons, the AHS (2014) does not recommend the use of “slow-kill” therapy in the management of HWD.
**Comparing treatment options.** Treatment of HWI with melarsomine dihydrochloride, in this article referred to as *adulticidal therapy*, differs from “slow-kill” treatment in many respects. As stated in the previous section, “slow-kill” therapy usually incorporates the administration of heartworm preventive, ideally in combination with doxycycline therapy. The AHS (2014) management protocol for HWI recommends that adulticidal therapy include administration of heartworm preventive to reduce microfilarial counts; prescription of doxycycline to kill endosymbiotic *Wolbachia*, thus reducing inflammatory pathology; ideally, three injections of melarsomine; and exercise restriction from the day of diagnosis to six to eight weeks following the last melarsomine injection. In other words, the AHS-recommended protocol for adulticidal therapy includes those protocols of “slow-kill” therapy with other additional procedures.

It follows that adulticidal therapy must have greater financial costs than “slow-kill” therapy. This comparison is noted in many studies surrounding HWD management (Colby et al., 2011; Polak & Smith-Blackmore, 2014), although objective comparisons of the average costs of HW treatment have not been performed. Additional financial costs may incur if diagnostic methods, such as the blood analyses and radiographs recommended by the AHS (2014), are required for understanding and treating the patient’s pathology. Non-monetary costs on pet owners should also be considered. Confining or restricting the pet’s activity for at least three months over the course of adulticidal treatment may be considered inconvenient by many pet owners. Additionally, adulticidal administration and diagnostic testing requires prolonged separation between pet and owner, including overnight hospitalization. Such separation can understandably
cause stress to both pets and their owners alike. A summary of HW treatment comparisons is provided in Table 1.

<table>
<thead>
<tr>
<th>Adulticidal Treatment</th>
<th>“Slow-Kill” Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic Agents</strong></td>
<td>Melarsomine Dihydrochloride (Immiticide®) + Doxycycline</td>
</tr>
<tr>
<td><strong>Safety &amp; Efficacy</strong></td>
<td>Only treatment recommended by the AHS due to empirical evidence for safety and efficacy</td>
</tr>
<tr>
<td><strong>Non-monetary Considerations</strong></td>
<td>Inconvenient: requires hospitalization, confinement, activity restriction; possible further clinical workup (e.g. bloodwork, radiographs)</td>
</tr>
<tr>
<td><strong>Monetary Considerations</strong></td>
<td>Expensive: $1,200-$1,800 + preventive cost</td>
</tr>
</tbody>
</table>

**Table 1. Comparing heartworm treatment options.** Monetary data for adulticidal treatment is estimated for a 40-lbs dog, provided by the AHS (2013). Monetary data for “slow-kill” treatment includes doxycycline cost, obtained from current estimates for 30 days of doxycycline for a 40-lbs dog in the practice included in this study.

**Understanding clinical decision-making.** Perhaps due to these factors, the “slow-kill” method remains a common practice despite AHS recommendations. However, few studies have explored its prevalence. A survey of animal shelters in HW-endemic states (Florida, Georgia, Alabama, and Mississippi) showed that 22% of the shelters
predominantly used “long-term low-dose ivermectin”, or “slow-kill” therapy, as HW treatment (Colby et al., 2011). In clinical practice, less than 10% of HW-positive cases in veterinary practices surveyed throughout the MS Delta region received “slow-kill” therapy, according to preliminary results from an ongoing study (Pulaski et al., 2016).

The aforementioned study emphasized the need to bridge academic and clinical environments in HW treatment, but also mentioned the possible effects of clinician opinions and biases on questionnaire analysis results (Pulaski et al., 2016). Indeed, while these retrospective analyses of medical records are important tools in observing owner compliance and patient histories, there are limitations in performing retrospective analyses of veterinarian and pet owner behavior. In a separate, unpublished study (Ku et al., 2016), I investigated the role that LOE case frequency may play in ML-resistance development in the MS delta through the retroactive analysis of client and medical records from a private practice in northern MS. Our study shows that data are difficult to obtain from practitioner self-report, and there is also a noticeable dichotomy between practitioner perspectives and recorded data.

In fact, most practices lack the time, appropriate software, or interest to consistently and accurately record the clinical justifications for each treatment decision. As a result, studies often rely on practitioner self-reporting by memory, and thereby have the potential to contain error or personal bias. In one study, even when owners and veterinarians believed that a patient had received HW preventive with “perfect” compliance, gaps of coverage were still detected in a high number of cases (Atkins et al., 2014). Accordingly, it is possible that the actual prevalence of “slow-kill” therapy could be much greater than that which is self-reported.
Research Objectives

The purpose of this study is to better understand current treatment decisions employed for HW-positive patients, protocols utilized in a “slow-kill” methodology, and trends in HW prevention in a region with concerns about ML resistance. The objectives, hypotheses, and predictions of this study are summarized in Table 2.
<table>
<thead>
<tr>
<th>Objective</th>
<th>Hypothesis</th>
<th>Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 To determine the frequency of treatment types used for HW-positive canine patients and compare them to practitioner estimates</td>
<td>Practitioners typically under-report the frequency of “slow-kill” therapy used in their practices.</td>
<td>Practitioner estimates for the percentage of HW-positive cases treated with “slow-kill” therapy will be less than the actual percentage revealed by patient medical records in this practice.</td>
</tr>
<tr>
<td>2 To survey practitioner opinion on the factors influencing HWD management choices</td>
<td>Financial concerns are a primary factor influencing client and veterinary treatment decisions for patients in this region.</td>
<td>Veterinarians will report that clients prefer to use the “slow-kill” method of HWD treatment due to financial concerns.</td>
</tr>
<tr>
<td>3 Analyze HWP history for HW-positive patients in this practice in order to understand the causes of HW infection in this region</td>
<td>Lack of compliance to existing HWP is the primary cause of HWI in this region.</td>
<td>Most HW-positive patients will demonstrate poor histories of HWP.</td>
</tr>
</tbody>
</table>

Table 2. Research objectives, hypotheses, and predictions.
CHAPTER 2

METHODS

Participants

The study was performed at a mixed-animal private practice in Oxford, Mississippi, that employs four veterinarians. Oxford falls within the region of high density for lack-of-efficacy claims (Hampshire, 2005). From April to June 2016, canine patients were tested for HWI using the SNAP HW RT or 4Dx Plus antigen tests (IDEXX Laboratories, Westbrook, ME, USA) during routine annual examinations or when experiencing appropriate clinical signs, at a doctor’s discretion. This test method was chosen primarily because it was the existing HW diagnostic test utilized at the participating practice. The SNAP HW RT test has also been shown to be significantly more sensitive than other commercial heartworm antigen test kits of its type (Atkins, 2003). The SNAP test, like all HW antigen tests, can indicate HWI as early as six months after transmission. Dogs testing positive for HWI were recorded for further analysis of their medical records.

Data Collection

For HW-positive cases, client transaction records and patient charts were scrutinized for HW preventive purchase gaps, purchases for multiple patients in the same household, and patient HW testing and/or treatment history. Prevention purchases for
multiple patients in the same household were included in the criteria because product sharing may indicate possible compromises in HW protection (Atkins et al., 2014). When available, heartworm preventive history was studied from two years before positive testing, or from birth if the patient was less than two years old. Patients were assigned a status under the following criteria (summarized in Table 3):

a. Consistent – no gaps in coverage greater than three months; such gaps in coverage may be realistically accounted for by the retroactive efficacy (“reach-back”) of some preventive products (McCall, 2005)

b. Inconsistent – gaps in coverage greater than three months

c. None – no record of preventive use

d. Unknown – patients without available medical records over the period of interest (i.e. newly adopted pets)

The treatment protocols chosen for these heartworm-positive dogs were recorded in one of three categories: adulticidal therapy, indicating the administration of melarsomine (Immiticide®, Merial Limited, Duluth, GA); “slow-kill” method, indicating the off-label use of an ML preventive as an adulticide for active HW infection in addition to at least one month of doxycycline; and no treatment. The type of ML preventive used for “slow-kill” therapy cases was also recorded in each case.
For heartworm (HW)-positive patient…

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Does client purchase history exist for this patient?</td>
<td>Continue to #2</td>
</tr>
<tr>
<td>2</td>
<td>Has client purchased HW prevention in the two years prior to (+) HW test?</td>
<td>Continue to #3</td>
</tr>
<tr>
<td>3</td>
<td>In this period, is there a gap in HW prevention greater than three months?</td>
<td>INCONSISTENT</td>
</tr>
</tbody>
</table>

Table 3. Heartworm prevention history decision tree.

Practitioner and Client Opinions

Veterinarians were asked to complete a questionnaire regarding their methods of and beliefs with respect to HW treatment protocols. They were asked to estimate the percentage of HW-positive dogs diagnosed in this practice that they believed received “slow-kill” therapy and whether they began discussions with clients regarding HW treatment by introducing adulticidal therapy or “slow-kill” therapy. They were also asked to indicate and rank the primary factors that they believe influence clients to choose “slow-kill” over adulticidal methods of HW treatment, and to expand on these reasons if possible.

Data Sorting and Analysis

This practice utilized both digital and physical (paper) medical records, so all records were pulled from AVImark® software (Logistic, 2009) or physical patient files and recorded using Microsoft® Excel (Microsoft Office, 2016).
(WOI) analyses were performed for patients with a consistent history of HW preventive use in the previous two years using the Merial© Window-of-Infection program (www.heartwormedu.com). The window of infection is defined as the period of time in which the current infection is most likely to have occurred. This time period starts at nine months prior to the last negative HW test and ends six months before the positive HW test. Purchase gaps of 45 days or more within the WOI indicate compliance failure, and argue against product failure (Atkins et al., 2014).
CHAPTER 3

RESULTS

Heartworm Prevention Histories

Of 321 dogs tested for HW over the period of this study, a total of 40 dogs tested HW-positive (12.46%). Client records revealed that over half of all HW-positive patients had inconsistent (32.5%, n=13) or no history (30.0%, n=12) of HW prevention in the past two years (Figure 4). The remaining cases consisted largely of patients with an unknown HW prevention history (27.5%, n=11). Few patients had consistent HW preventive coverage (10.0%, n=4); one such patient had previously tested positive and was currently under “slow-kill” treatment. Window-of-infection (WOI) analyses were performed for the remaining three patients that appeared to have a consistent preventive history. These analyses identified preventive purchase gaps of >45 days within the WOI for all three of the HW-positive patients considered (Figure 5). It is of interest that all three dogs showed good compliance after the first purchase of medication, but were infected prior to having received heartworm prophylaxis. There were two LOE claims submitted to pharmaceutical companies for compensation during this period; both claims were submitted from the patients who were considered to have Consistent HW preventive coverage.
Figure 4. Prevention history for heartworm-positive patients. The shaded region (a) in the first column denotes the proportion of cases that were determined to have consistent HW prevention coverage were not found to have purchase gaps >45 days after WOI analysis (2.5%).
Figure 5. Window-of-infection analyses for three heartworm-positive patients. In these diagrams, a white circle represents a single heartworm preventive dose, an asterisk indicates the purchase of additional preventive medication, and a positive or negative symbol denotes the time and result of heartworm testing for the patient in question. These dogs (a, b, c) appeared to have consistent heartworm prevention coverage during initial scrutiny of patient records, but gaps >45 days were apparent in the WOI analyses. These gaps are symbolized by red coloration of the horizontal timeline.
Heartworm Treatment Methods

Results for each heartworm treatment method are summarized in Table 4 and Figure 6. Of the HW-positive patients in this study, a majority received treatment using a “slow-kill” method (75.0%, n=30). Patients were equally likely to receive an arsenical (melarsomine) as no treatment (12.50%, n=5). Of the five patients who received no treatment, two were experiencing severe health complications and were euthanized before HW treatment could be considered. Another was in the care of a rescue group and was transferred to a different organization before HW treatment was considered. The remaining two patients received no HW treatment due to client decisions.

Four different HW preventive choices were utilized for “slow-kill” treatment in this practice: injectable moxidectin (ProHeart®, Pfizer Inc., Madison, NJ), topical moxidectin/imidacloprid (Advantage Multi®, Bayer Animal Health, Shawnee, KS), oral milbemycin oxime/spinosad (Trifexis®, Elanco Animal Health), and oral ivermectin/pyrantel (Heartgard® Plus, Merial Inc., Duluth, GA) (See Figure 3). Of these products, a majority (83.33%, n=25) of cases were treated with injectable moxidectin. Oral ivermectin (6.67%, n=2), milbemycin oxime (6.67%, n=2), and topical moxidectin (3.33%, n=1) were chosen far less in “slow-kill”. Doxycycline therapy was prescribed for 85% (n=25) of patients treated with “slow-kill”; for the remaining patients, clients declined doxycycline therapy.

<table>
<thead>
<tr>
<th>Month</th>
<th>Total HW+</th>
<th>Immiticide</th>
<th>Slow Kill</th>
<th>No Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>May</td>
<td>15</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>June</td>
<td>17</td>
<td>1</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>5</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4. Sample sizes for each heartworm treatment.
Figure 6. Heartworm treatment decisions and macrocyclic lactone preventives chosen for “slow-kill” treatment.

Treatment Methods Questionnaire

Practitioner questionnaire results are depicted in Figure 7. When asked to estimate the treatment decisions regarding their HW-positive canine patients, these veterinarians believed on average that 53.75% of HW-positive dogs in this practice received “slow-kill” therapy (instead of adulticidal treatment or no treatment), although estimates ranged from 10.00% to 75.00%, depending on the practitioner (Figure 7a). Each of the practitioners reported that when discussing HW treatment options with clients, they introduced adulticidal therapy before “slow-kill” therapy options (Figure 7b).

Factors influencing “slow-kill” therapy choice are shown in Figure 7c. The practitioners cited client financial concerns as the primary deciding factor for clients who chose “slow-kill” therapy (78.3%) since adulticidal therapy requires additional charges for the drug, drug administration and hospitalization. The second-most commonly cited
factor was convenience (14.0%) as many clients disliked the confinement aspect of adulticidal therapy, particularly in cases involving active, asymptomatic patients. Patient age was another influential factor in HW treatment considerations, as clients often objected against stressful or expensive treatment procedures for older canines. Arsenical concern (2.33%) and preexisting life-threatening disease (2.33%) were less common reasons that clients chose “slow-kill” therapy.
(a) Estimate the percentage of dogs diagnosed with HWI that receive slow/soft-kill therapy in this practice under your care.

A comparison between the average practitioner estimate and the actual value obtained in study is depicted.

(b) When discussing protocols for heartworm treatment with clients, which adulticide method do you usually begin your discussion with?

Practitioners could indicate one of two choices.

(c) Why do clients choose “slow-kill”? Please indicate the relative importance of each factor.

**Figure 7a-c. Practitioner questionnaire and results.** (a) The mean practitioner estimate is compared with the actual percentage of HW-positive cases treated with “slow-kill” therapy in this practice during the study period. The red dots represent the values of individual estimates (10.0%, 60.0%, 70.0%, 75.0%). Practitioners underestimated the prevalence of “slow-kill” therapy in this study.
CHAPTER 4
DISCUSSION

Causes of Heartworm Infection

Heartworm infection in this practice resulted most often from inadequate (32.5%), unknown (27.5%), or absent (30.0%) ML preventive use. Even in the 10% of HW-positive dogs that consistently received ML, deeper analysis revealed purchase gaps of 45 days or greater in three of four cases. Thus, 97.5% of HW-positive patients in this study had inadequate HW protection. In a study of HW-positive cases suspected to be a result of ineffective HWP, 80.7% of patients were found to have inadequate HWP coverage (Atkins et al., 2014). Since the Atkins et al. (2014) study contained a more specific study group in which consistent HWP was expected, it is not surprising that they would have a lower percentage of HWP incompliance than that of our study. Thus, our results do not depart from existing research on HW-positive samples.

Furthermore, LOE claims were submitted for only two (0.62%) of the 321 dogs tested during this time period. This is consistent with previous observations from an epidemiological study exploring the factors that may have contributed to an increase in LOE, showing that, over the past 10 years, annual LOE cases made up ≤1.3% of total HW tests performed (Ku et al., 2016). An existing survey of the MS delta reports that while a large number of practices (26%, n=57) have only seen one to two cases of well-documented HWP failure in the last year, some practices (16%, n=35) also report having
seen 10-50 cases of HWP failure in the past year (Pulaski et al., 2014). Thus, there is a wide range of possible LOE caseloads in the MS delta region, and the data from our study is consistent with this range.

Do these LOE cases indicate the possibility of HW resistance in this practice? As previously stated, gaps in HW protection were still detected in the two cases submitted for LOE consideration. LOE claims may be incorrectly assumed to indicate suspected product failure or HW resistance to ML products, especially since increased LOE cases in the past decade have raised concerns of HW resistance (Bowman, 2012). However, LOE cases may be submitted in any circumstance where patients test HW-positive while receiving HWP. Pharmaceutical companies may compensate practitioners or clients in cases of alleged product failure, providing an incentive for practitioners to submit LOE claims regardless of known gaps in HW protection. Even in cases of inadequate HWP, pharmaceutical companies may still provide compensation because some HW preventives are advertised to have retroactive or “reach-back” effects that may prevent HWI even in the case of missed doses (McCall, 2005). Pharmaceutical companies and the FDA Center for Veterinary Medicine have adopted several scoring systems for LOE claims that evaluate the possibility of drug ineffectiveness, but reports of LOE frequency often neglect the distribution of such scores, resulting in misleading numbers (Hampshire, 2005). Thus it is invalid to assume that LOE claims equate to cases of resistance since HW protection is often found to be inadequate for the patients under consideration (Atkins, 2014).
Practitioner Perceptions and Heartworm Management Practices

In this practice, the majority of HW-positive cases were treated with “slow-kill” therapy during the months of this study. These data contrast preliminary results of questionnaires in the MS delta region, where practitioners reported “slow-kill” therapy usage in less than 10% of HW-positive cases (Pulaski et al., 2016). The in-clinic survey results (Figure 4a) from this study show that although these practitioners have higher estimates than other veterinarians in the MS delta region, they still underestimate the use of “slow-kill” therapy in their own practice (with a percent error of 28.3% between estimate average and actual value). These results offer support for our first hypothesis, that practitioners typically under-report the frequency of “slow-kill” therapy used in their practices.

There is a large disparity between the estimates made by different practitioners in the clinic, with the greatest outlier being one practitioner’s very low estimate of only 10% of cases being treated with “slow-kill”. Possible explanations for this disparity include differences in individual HW treatment decisions, variable exposure to HWD management due to the division of practitioner duties within the clinic, and personal beliefs toward the adoption of “slow-kill” methods. The impact of differences in individual HW treatment decisions of each practitioner should be reduced in the analysis of overall HW treatment decision outcomes, as the mean of all practitioner estimates should comprise the outcome in the practice as a whole. The practitioner who made the lowest estimate for “slow-kill” practices in surgery a majority of the time, where heartworm management is less frequently encountered. Additionally, personal communications with this practitioner revealed that he places considerable importance on
the possibility of unsafe complications that may be caused by “slow-kill” treatment, and thus usually invests more resources into persuading clients to pursue adulticidal therapy. Although it is likely that this practitioner still underestimates his utilization of “slow-kill” despite his beliefs, this cannot be confirmed in this study. It would be beneficial for future analyses of clinical heartworm management decisions to address the impact of individual roles and beliefs on self-report by including practitioner-specific comparisons of estimates and actual behavior. However, this may prove difficult if patient cases are seen by multiple doctors, as treatment decisions may not be exclusive of other practitioners in the clinic.

The results of this comparison, along with the generally low return rate on surveys, underscore concerns that practitioner questionnaires may be inaccurate tools for such data collection. However, one of the major limitations of this study is its small scope, as it enrolled only a single practice with four veterinarians and analyzed only three months’ activity, which is not a representative sample of veterinary practices. Thus, the results of this study cannot be generalized to other practices in the encompassing region. In reality, such extensive review of medical records is often impractical for the majority of veterinary practitioners. This is due to time constraints, digital medical recording practices which are not designed for retrieving such information, and paper medical records that are cumbersome and time-consuming to search through. Perhaps most significant is the fact that many medical records retain inadequate data to answer the questions of research. Thus, while small studies such as this one can provide a unique, deeper insight into HW disease management, self-report questionnaires, despite their
flaws, remain an important and efficient tool for investigating and understanding clinical behavior in veterinary practices.

Another limitation of this study is the testing protocol used to determine heartworm-positive dogs in the study group, as it may have posed a limit on the numbers of HW-positive cases included in analysis. As previously stated, HW testing was done through the Idexx SNAP HW RT protocol, which utilizes ELISA to detect an antigen produced by the adult female heartworm. Although this test has a sensitivity of as much as 90% and is significantly more sensitive than other HW immunoassay products (Atkins, 2003), false negatives are still possible for several reasons. Due to the long duration of the *D. immitis* life cycle from vector-transmitted larval stage to adult, this testing protocol can only detect HWI around six months after the onset of infection; in addition, worm burdens low in or absent of female worms will not produce detectable antigen, resulting in a false negative or misleading low antigen report (Atkins, 2005). For these reasons, the American Heartworm Society (AHS, 2014) advocates that practitioners include microfilarial testing, or direct observation of blood samples for existing microfilaria, in combination with antigen testing to detect HW in infected dogs with antigenemia.

Recent studies have also demonstrated the role of antigen-antibody complexes in confounding antigen testing. In some studies, canine blood samples testing seronegative for HW antigen have been shown to test seropositive after the samples are heated; the researchers conducting these studies have proposed that heat treatment improves diagnostic accuracy of HWI since certain patients, such as those currently receiving ML preventive, may have immunologically inhibited *D. immitis* antigen during initial stages of infection (DiGangi et al., 2016; Savadelis et al., 2016; Valesquez et al., 2014).
However, the role of antigen-antibody complexes and their validity in representing active HWI is still disputed and requires continued research.

As a result of these limitations, there may have been a greater number of positive dogs served in the practice than reported in this study. In order to overcome these limitations, future studies in this or other practices should employ microfilarial testing in addition to antigen testing protocols in order to decrease false negatives and provide a more accurate representation of HW prevalence. The AHS (2014) does not currently recommend heat treatment of patient serum for HW antigen testing because this would represent an off-label use of available antigen tests; regardless, it is possible that heat treatment could also increase sensitivity of HW diagnoses.

The ML preventive that was chosen for use in a majority of “slow-kill” treatments in this practice was injectable moxidectin. This may be due in part to the nature of this preventive and its decreased dependence on client compliance. Administration of injectable moxidectin is performed in the practice by a veterinary professional instead of by the client at home, and it requires less frequent administration (every six months) than other HWP products used in this practice (typically every month). When owners are responsible for administering monthly HWP, such as in the case of oral and topical products, there is rarely a reliable record of proper compliance (e.g. every 30 days). It is possible that pet owners may delay doses of medication or skip them entirely, permitting a window of infection in which the preventive is not 100% effective, and either overlook the lapse in coverage or choose not to report their mistakes. In contrast, administration of injectable moxidectin is reliably recorded through patient records and client purchase histories. Additionally, one veterinarian at this practice stated that since owners and their
pets must routinely return to the clinic for administration of injectable moxidectin, there are more opportunities to routinely examine the patients and a greater likelihood of regular HW testing. In support of this phenomenon, Zoetis, the manufacturer of the only currently available injectable moxidectin product, sponsored a study that found that veterinarians were able to address additional medical issues in 21.7% of patients who had presented to animal hospitals for administration of injectable moxidectin (Lavan & Login, 2015). Most importantly, injectable moxidectin seems to ensure the successful administration of HWP without lapses in coverage.

As previously stated, the AHS currently recommends against the off-label use of any ML preventives as an alternative adulticide therapy for HWI-positive patients. Despite this, studies have recently been performed to explore the efficacy of topical moxidectin/imidacloprid as an adulticide in combination with doxycycline. These ongoing studies have had promising results, indicating efficacy levels as high as 95.9% (Savadelis et al., 2016). Researchers presenting these studies have proposed topical moxidectin and oral doxycycline combination therapy as a safe alternative to melarsomine adulticidal therapy (Savadelis et al., 2016; Ames et al., 2016; Genchi & Kramer, 2016). While these studies explore the efficacy of topical moxidectin, the practice in this study primarily utilized injectable moxidectin, along with oral doxycycline in 85% of “slow-kill” cases. While moxidectin may have similar mechanisms of action despite different administration methods, further study would be necessary in confirming the efficacy of the drug in combination with the benefits of injection administration.
Factors Influencing Heartworm Disease Management

The questionnaire results in this study reveal that clients who elect “slow-kill” therapy for their HW-positive dogs are primarily influenced by financial factors. As it has been previously stated that the validity of self-report should be scrutinized, this deserves further investigation beyond practitioner opinion. In fact, the importance of financial factors is supported by the state of the financial environment that pervades the MS delta region: that is, a significant percentage of clients in this region of high-density canine HW cases experience severe poverty. According to US Census data from 2010-2014, the county served by the practice in this study has a median annual household income ($41,343) much lower than that of the United States ($53,482) and a poverty level (26.1%) higher than than of the United States (15.6%). Concurrently, many states included in the MS delta region have low household incomes and high poverty levels compared to the national average (US Census, 2015). As previously discussed, the cost of recommended HW treatment is higher than that of “slow-kill” treatment. According to an informal practitioner survey (AHS, 2013), the average cost of heartworm treatment for a 40-lbs dog ranges from $1200-1800, which is 2.9-4.35% of the median household income in this region. Considering the importance of financial factors impacting HW management in the clinical environment and the results of academic inquiry, it may be beneficial to recognize the utility of “slow-kill” therapy in HW management, especially when it is impossible to mitigate the costs of melarsomine therapy. However, the lack of data concerning the actual costs of heartworm treatment methods is notable, and cost analyses would constitute an important step in understanding the financial aspects of heartworm treatment in order to develop more cost-efficient therapies. The results of this
study along with the relationship between economic trends and heartworm epidemiology suggest that these monetary factors play a significant role in HW management decisions and should not be overlooked.

As previously stated, the costs of HWI extend beyond monetary considerations. HWI alone introduces irreversible pathology to the lungs and cardiovascular system of dogs, potentially limiting the pet’s lifespan (Atkins, 2005). The recommended HW treatment involves hospitalization, confinement, and restricted activity, resulting in the emotional stress of separation to both pet and pet owner. Some researchers have suggested that “slow-kill” combination therapy should be preferred over melarsomine therapy because they do not require exercise restriction (Savadelis et al., 2016; Ames et al., 2016), which is notable regarding this study because practitioners cited the inconvenience of confinement as the second-most important factor influencing clients’ HW treatment decisions. Although cost analyses between HW treatment therapies are scarce, results from the American Heartworm Incidence Survey report that HW prevention costs are typically less than ten percent the cost of HW treatment (AHS, 2011). Thus, maintaining good HW prevention protocols and increasing client compliance to existing HW preventives remains an essential and cost-effective method of decreasing HWI, along with protecting patients from HW pathology.

Conclusions

My study offers perspectives on the prevalence of “slow-kill” therapy for treatment of HWI and the discrepancies between medical records and practitioner opinions. The evidence from 40 HW-positive cases shows that the “slow-kill” method is a prevalent
HW treatment choice of clients and veterinarians despite AHS recommendations. Injectable moxidectin is the ML preventive of choice for “slow-kill” therapy in an overwhelming number of cases. Practitioner questionnaires reveal client financial concerns as the primary factor driving HW treatment towards “slow-kill” therapy and away from melarsomine therapy, although convenience also remains an important factor. As cost and convenience levels of current melarsomine adulticidal procedures may be impractical to change, there is a need for greater study on the efficacy and risks of the “slow-kill” method and the effects of different ML preventives and combination therapies for this off-label use. Such research for injectable moxidectin should be a particular priority in light of its prevalence in this study, the benefits of its administration method, and the recent studies regarding the efficacy of topical moxidectin.

Comparison between these medical records and the estimates reported in the questionnaires also reveal discrepancies between practitioner opinion and the reality of HW management decisions in that “slow-kill” therapy is far more prevalent than estimated. While self-report questionnaires remain an important and useful research tool, additional research should be conducted to explore alternative methods of data collection for clinical management investigations to continue bridging the gap between academic and clinical environments of HW disease management. For example, it would be beneficial to implement a clinical survey to determine the success rate of “slow-kill” therapy in reverting HW-positive antigen tests within practices that have already elected to use “slow-kill”. Ideally, participating practices would have a large sample size of HW positive canines undergoing this form of treatment. Such a study could provide information on clinical success rates for different HW preventives, estimates for average
length of administration required for remission, and documentation of possible risks and side effects, greatly adding to the current understanding of “slow-kill” efficacy and risks. Additionally, while case studies such as this one are a useful tool, development of software allowing more efficient yet accurate data collection from multiple practices would serve extremely useful as well.

Ultimately the data, in addition to WOI analyses, suggest that poor client compliance with HW preventive administration remains the predominant cause of HW infection. This suggests that practicing veterinarians should take efforts to ensure client compliance with existing, effective HW preventatives in order to reduce HW disease prevalence, regardless of local parasite resistance. While heartworm resistance to MLs is certainly deserving of continued study, lack of compliance to existing HWP protocols must first be addressed in order to provide a more accurate understanding of the impact of resistance.
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