Efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) for the prevention of heartworm disease and infection all month long

Dwight D. Bowman1,3*, Cameon M. Ohmes2, Joseph A. Hostetler2, Daniel J. Keil2, Terry L. Settje2 and Samuel D. Charles2

From 15th American Heartworm Society Triennial Symposium
New Orleans, LA, USA. September 11-13, 2016

Abstract

**Background:** Prior work has shown that the levels of moxidectin in dogs treated with Advantage Multi® for Dogs (Bayer Animal Health) remain at a high plasma concentration for the full month after application. The objective of this study was to demonstrate the efficacy of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs, also known as Advocate® for Dogs) for the prevention of heartworm infection and disease 30 days after just one application.

**Methods:** Two groups of eight dogs each were included. Dogs in Group 1 received the product (Advantage Multi® for Dogs) while those in Group 2 remained as nontreated controls. All dogs entering the study completed a physical examination including examination for *Dirofilaria immitis* antigen and circulating microfilariae. Dogs in Group 1 were treated on Study Day (SD) –30 as per the label recommendation. Thirty days later (SD 0) dogs in Groups 1 and 2 were subcutaneously infected in the inguinal region with approximately 50 infective third-stage *D. immitis* larvae (“Missouri” isolate). Blood was collected on SDs 120 and 147 for examination for *D. immitis* antigen and circulating microfilariae. On SD 148, all animals were euthanized and necropsied for recovery of adult heartworms. All procedures were performed in accordance with the VICH GL9 guidelines.

**Results:** Examination and worm counts made at necropsy showed no heartworms in the treated dogs (Group 1) compared with six of eight nontreated dogs (Group 2) with heartworms (range of 2–33). The treated dogs (Group 1) had significantly fewer heartworms (*p* < 0.05) compared with the nontreated controls (Group 2).

**Conclusion:** The results demonstrated that 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) is efficacious for the prevention of heartworm infection and disease all month long with no observation of treatment-related adverse events.

**Keywords:** Advantage Multi® for Dogs, *Dirofilaria immitis*, Heartworm disease, All-month protection, Forward protection, Persistent efficacy, 30-Day protection, Proactive prevention, Imidacloprid, moxidectin, Advocate®
Background
Persistent efficacy of topical imidacloprid + moxidectin (Advantage Multi®, Bayer Animal Health) was first demonstrated in dogs and cats against hookworm infections. In 2003, it was shown that when eight dogs were administered 10% imidacloprid + 2.5% moxidectin topical solution and subsequently infected with 300 Uncinaria stenocephala 18 days later, neither immature nor mature adult hookworms were present 21 days after infection, while the eight nontreated dogs harbored a mean of 4.6 (SD ± 3.9) immature adults and 8.1 (SD ± 4.3) mature adults [1]. In 2008, a similar study with hookworms of the genus Ancylostoma was reported in which cats and dogs were respectively administered each month, as per label, either five monthly treatments of 10% imidacloprid + 1% moxidectin topical solution (Advantage Multi® for Cats) or four monthly treatments of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) [2]. Twenty (20) days after the last treatment, the six treated and six nontreated cats were orally infected with Ancylostoma tubaeforme larvae. Necropsy examinations conducted after establishment of patent infections in the nontreated cats showed none of the six treated cats had hookworms, while a mean of 62.5 adult A. tubaeforme was recovered from the nontreated cats. Each of the six treated and six nontreated dogs were orally infected with U. stenocephala larvae and Ancylostoma caninum larvae 22 and 23 days, respectively, after the fourth treatment. Necropsy examinations conducted after establishment of patent infections in the nontreated dogs showed all six treated dogs had no adult U. stenocephala, while a mean of 517.8 adult U. stenocephala was recovered from the nontreated control dogs. One adult A. caninum was recovered from one treated dog, while a mean of 41.3 A. caninum was recovered from the nontreated dogs.

Two studies evaluated the persistent efficacy of the pretreatment of cats and dogs with topical imidacloprid + moxidectin against heartworm infection. A study with 19 cats examined the effects of four monthly treatments (at 28-day intervals) followed by infection of each cat with 25 infective-stage Dirofilaria immitis larvae at 7, 14, 21, and 28 days after the last treatment [3]. Seven and one-half months after the fourth monthly treatment there were no heartworms recovered at necropsy from any of the 10 treated cats while a mean of 1.33 heartworms (range 1–6) was recovered from the nine nontreated cats. A study with a similar design was performed in 16 dogs (eight treated and eight nontreated dogs) in which the treated dogs had received four monthly treatments (at 28-day intervals) and then were infected with 50 third-stage D. immitis larvae 28 days after the fourth monthly treatment [4]. Again, there were no heartworms recovered from any of the treated dogs at necropsy conducted 5 months post infection, while a mean of 33.9 (range 25-41) heartworms was recovered from the eight nontreated dogs. These two studies demonstrated that four monthly applications of imidacloprid + moxidectin topical solution afforded protection to dogs and cats against subsequent heartworm infection for 4 weeks.

Previous publications evaluating the pharmacokinetic profile of moxidectin demonstrated that the repeated monthly application of topical imidacloprid + moxidectin to dogs and cats produces a continuous high level (steady-state) of protective moxidectin, month to month, after four or five repeated doses and also appears to provide protection for at least 28 days after the last administration against hookworms and heartworms in dogs and cats [2–4]. In the above referenced dog study, the authors postulated that due to this unique pharmacokinetic profile and high serum concentrations protection for 30 days against heartworms after just one dose is probable. The objective of this study was to determine whether a single application of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) would protect dogs against an incoming D. immitis larval challenge 30 days after a single topical application of the product.

Methods
Ethical approval, animals, and animal care
The study was approved by the facility Institutional Animal Care and Use Committee (IACUC) with the concurrence of Bayer Animal Health and followed the guidelines of VICH GL9 (2001) [5]. The study included 16 purpose-bred mongrel dogs (six males and ten females) that were born and raised at the study facility that were approximately 7 months old at the beginning of the study. The dogs were maintained in indoor runs during the study. Nontreated dogs were housed in a separate room so that there was no contact with the treated dogs. Temperature was controlled by forced fan heat or air conditioning as needed. The dogs were fed a daily ration of Laboratory Canine Diet 5006 (LabDiet) in quantities sufficient for growth and maintenance. Dogs were provided water, originating from the municipal water supply, via individual automated watering system in each cage. All dogs were observed once daily as part of a general health observation with the exception of the day of treatment, Study Day (SD) –30, when the dogs were observed at 0.5, 1, 2, 4, and 8 h (+/−15 min) post treatment. Animals were weighed on SD –31 for ranking, randomization, and dose determination for SD –30.

Blood samples were collected from the study animals on SD –37, 120, and 147 for D. immitis antigen and microfilariae testing. Antigen testing was performed using the
DiroCHEK® heartworm antigen test (Zoetis). Microfilariae testing was performed using the modified Knott test.

Treatment
Using the body weights collected on SD –31, the 16 dogs were randomized to two treatment groups. Eight dogs in Group 1 were treated with 10% imidacloprid + 2.5% moxidectin topical solution as per label instructions and dosage recommendations on SD –31, while eight dogs in Group 2 remained untreated (Table 1).

Randomization
Dogs were randomized by pretreatment body weight. Sixteen (16) dogs meeting the inclusion criteria were allocated to two study groups of eight dogs each on SD –31. Dogs were assigned to study groups according to a predefined randomization chart. The dogs were ranked by SD –31 body weights in descending order (highest to lowest). The animal ID was used to break ties (highest to lowest number). The first two dogs (heaviest) were assigned to block 1, the next two dogs assigned to block 2, and so forth, until the final two dogs (lowest body weights) were assigned to the final block. Within each block, the randomization chart was created such that each dog within each block had equal chance at being assigned to one of the two treatment groups.

Masking
Randomization of dogs to study groups and administration of the 10% imidacloprid + 2.5% moxidectin topical solution was performed by an unmasked designated person at the study facility. Witnesses to these procedures were also not masked. After randomization, the designate and witnesses were not actively involved in any other experimental procedures. All other people involved in the study execution were masked to the study group allocation.

Inoculation with heartworm larvae
The study animals were inoculated subcutaneously on SD 0 with 50 infective, third-stage (L3) *D. immitis* (Missouri isolate) harvested from infected mosquitoes (*Aedes aegypti*; Liverpool strain). This susceptible heartworm isolate originated from a naturally infected dog from northwest Missouri with no known history of treatment with macrocyclic lactones (Personal communication, Dr. Byron Blagburn, Auburn University, 2017). A blood sample positive for *D. immitis* microfilariae was originally collected from the donor dog on July 13, 2010 and was used to infect mosquitoes. The isolate was first validated in April 2011 via microfilarial testing, antigen testing, and heartworm recovery and was maintained at the College of Veterinary Medicine, Auburn University at the time of this study (Personal communication, Dr. Byron Blagburn, Auburn University, 2017). The infective third-stage larvae used for this study were harvested from mosquitoes 16 days after membrane feeding on a heparinized blood sample collected from a dog at Auburn University.

Postmortem examination
Study animals were humanely euthanized on SD 148 with Beuthanasia-D® [Schering-Plough Animal Health (now Merck Animal Health)] administered intravenously, 148 days after infection with third-stage *D. immitis* larvae. At necropsy adult heartworms were recovered, identified to gender, and counted. For heartworm collection, the right atrium was dissected through the main pulmonary artery. Next, each branching artery was dissected down to their furthest extent in each lung lobe. Finally, the other three heart chambers were dissected and examined for recovery of heartworms.

Statistics for efficacy determination
Descriptive statistics (number of animals positive, geometric mean and arithmetic mean heartworm counts per animal per group) were calculated for the *D. immitis* burdens of all study groups. The heartworm counts were used to evaluate the efficacy of the 10% imidacloprid + 2.5% moxidectin topical solution (the Investigational Veterinary Product, IVP) against *D. immitis*. Percent efficacy was calculated as follows:

\[
\text{Percent Efficacy} = \left(1 - \frac{N_2}{N_1}\right) \times 100
\]

\(N_1\) = Geometric mean count of *D. immitis* for Group 1 treated with IVP.

| Table 1 Treatment information on study animals in the 10% imidacloprid + 2.5% moxidectin (Advantage Multi®) and nontreated groups |
| --- |
| Treatment group | Gender | Dog ID# | Study day | Body weight (pounds) | Dose (mL) |
| --- | --- | --- | --- | --- | --- |
| Group 1: 10% imidacloprid + 2.5% moxidectin | Female | 1531603 | –30 | 29.0 | 2.5 |
| | Female | 1531805 | –30 | 16.6 | 1.0 |
| | Female | 1531806 | –30 | 18.6 | 1.0 |
| | Female | 1531903 | –30 | 19.0 | 1.0 |
| | Male | 1531502 | –30 | 21.0 | 2.5 |
| | Male | 1531801 | –30 | 25.4 | 2.5 |
| | Male | 1531802 | –30 | 24.2 | 2.5 |
| | Male | 1531901 | –30 | 30.6 | 2.5 |
| Group 2: no treatment | Female | 1531503 | –30 | 17.6 | 0 |
| | Female | 1531702 | –30 | 25.2 | 0 |
| | Female | 1531706 | –30 | 25.0 | 0 |
| | Female | 1531803 | –30 | 19.6 | 0 |
| | Female | 1531804 | –30 | 17.8 | 0 |
| | Female | 1532203 | –30 | 19.0 | 0 |
| | Male | 1531501 | –30 | 32.4 | 0 |
| | Male | 1532101 | –30 | 30.4 | 0 |
N2 = Geometric mean count of *D. immitis* for the nontreated Group 2.

In accordance with the VICH GL19 guidelines, the following criteria were to be met to confirm effectiveness of the IVP for heartworm treatment and prevention.

a. A minimum of six infected nontreated dogs (at least five adult heartworms recovered at necropsy per dog to substantiate adequate infection). Because this requirement was not met, a minimum of six infected nontreated dogs with at least one adult heartworm at necropsy per dog was considered as adequate infection.

b. Percent efficacy for the IVP group must be 100%.

c. A statistically significant difference in the number of heartworms at necropsy was needed between the treated group (Group 1) as compared with the nontreated group (Group 2).

A non-parametric statistical analysis (Wilcoxon’s Rank Sum Test) was used to test for group differences in heartworm counts using a 5% significance level. SAS Statistical Software version 9.3 was used to analyze the data from this study.

**Results**

**Efficacy of treatment**

Adult heartworms were not recovered from any of the eight 10% imidacloprid + 2.5% moxidectin topical solution–treated dogs in Group 1. Adult heartworms were recovered, however, from six of the eight nontreated dogs in Group 2. Overall for the nontreated dogs, a total of 134 adult *D. immitis* (range = 0–33 per dog) were recovered at necropsy (Table 2); the geometric mean *D. immitis* count for this group was 8.0 heartworms and the arithmetic mean was 16.8 heartworms (Table 3). Thus the nontreated group’s infection was considered adequate for determining efficacy of treatment. Percentage efficacy was determined to be 100%. No treatment-related adverse events were observed in any dogs.

**Heartworm antigen**

All blood samples collected on SD 7 and SD 120 were negative for the presence of *D. immitis* antigens, indicating no prior or unknown exposure to heartworm infection. On SD 147, one control dog, Dog #1531503, was positive on the heartworm antigen test; this dog had a total of 28 heartworms at necropsy. All dogs in both groups were negative for microfilariae at all three time points.

**Discussion**

In this study, a single dose of 10% imidacloprid + 2.5% moxidectin topical solution administered 30 days prior to heartworm infection was 100% efficacious in protecting the treated dogs from infection with this Missouri isolate of *D. immitis*. The results reported here were suggested as a possibility in an earlier publication with dogs that were treated four times every 28 days and then infected with this same isolate 28 days after the last treatment [4]. Similarly, research in cats treated with four topical doses of 10% imidacloprid + 1.0% moxidectin confirmed that after steady state was reached, all cats were protected all month long against incoming susceptible heartworm infective-stage larvae administered 7, 14, 21, and 28 days after the last treatment [3].

Moxidectin is a highly lipophilic macrocyclic lactone that is distributed and stored mainly in fat tissues [6] with a gradual elimination from the host. With the repeated application of topical 10% imidacloprid + 2.5% moxidectin for 4 months, moxidectin reaches a steady state and maintains a high plasma concentration between doses (mean concentration of 20 μg/L at 35 days after the last application) in comparison to the peak concentration after a single dose (15 μg/L) [4]. Due to the unique pharmacokinetics and high moxidectin serum concentrations maintained between monthly administrations of 10% imidacloprid + 2.5% moxidectin topical

---

**Table 2** Adult heartworms, *Dirofilaria immitis*, recovered 148 days after infection with third-stage larvae from eight dogs treated with 10% imidacloprid + 2.5% moxidectin (Advantage Multi®) 30 days prior to infection and from eight untreated control dogs

| Treatment group | Dog   | Adult *Dirofilaria immitis* |
|-----------------|-------|-----------------------------|
|                 |       | Males | Females | Total |
| Group 1: 10% imidacloprid + 2.5% moxidectin |       |       |         |       |
| 1531603         | 1531603 | 0     | 0       | 0      |
| 1561903         | 1561903 | 0     | 0       | 0      |
| 1531806         | 1531806 | 0     | 0       | 0      |
| 1531805         | 1531805 | 0     | 0       | 0      |
| 1531901         | 1531901 | 0     | 0       | 0      |
| 1531801         | 1531801 | 0     | 0       | 0      |
| 1531802         | 1531802 | 0     | 0       | 0      |
| 1531502         | 1531502 | 0     | 0       | 0      |
| Total worms recovered from treated dogs |       | 0     | 0       | 0      |
| Group 2: no treatment |       |       |         |       |
| 1531702         | 1531702 | 9     | 18      | 27     |
| 1531706         | 1531706 | 0     | 0       | 0      |
| 1531803         | 1531803 | 12    | 7       | 19     |
| 1532203         | 1532203 | 0     | 0       | 0      |
| 1531804         | 1531804 | 13    | 20      | 33     |
| 1531503         | 1531503 | 15    | 13      | 28     |
| 1531501         | 1531501 | 11    | 14      | 25     |
| 1532101         | 1532101 | 2     | 0       | 2      |
| Total worms recovered from control dogs | 62     | 72    | 134     |
| Mean worms recovered from control dogs | 7.8    | 9.0   | 16.8    |
solution [4], any heartworm infective-stage larvae entering the dog during the month after administration are likely to be prevented from developing or reaching the lungs to become adult worms. This is in contrast to other products with short half-lives that are rapidly eliminated from the animal, allowing for the establishment of an infection and the development of larvae between monthly doses [7, 8].

Not all heartworm preventive products are the same, especially in regards to resistant isolates, which can be attributed to the unique pharmacokinetic properties of topical moxidectin. In the work performed as part of the original approval for topical 10% imidacloprid + 2.5% moxidectin, a single treatment was 100% effective in protecting dogs against susceptible *D. immitis* strains [9]. Several other post-approval studies have evaluated the efficacy against resistant isolates with striking differences between products. For example, in one study, a single topical treatment of 10% imidacloprid + 2.5% moxidectin was 100% effective against the resistant JYD-34 isolate [9]; this was in contrast to an efficacy of 72% for milbemycin oxime (in NexGard Spectra®; Merial) even after six monthly treatments in a second study [10]. This killing ability, even against resistant isolates, is likely due to the distinctive properties of topical 10% imidacloprid + 2.5% moxidectin, which remains in the animal during the entire month between treatments so the animal is primed with drug at the time of infection. While a single topical treatment reaches plasma concentrations of 15 μg/L and has proven to be effective against multiple resistant strains [9], when an animal is maintained on monthly topical prevention with 10% imidacloprid + 2.5% moxidectin, a plasma concentration of ≥20 μg/L is maintained between monthly doses and extends for an entire month after the last application [4]. Therefore, there is every reason to suggest that dogs should be fully protected during this entire window against incoming susceptible and resistant heartworm isolates such as those shown to survive and develop following six monthly treatments with milbemycin oxime or other products that do not undergo steady-state phenomena [7–11]. If owners forget to give a monthly application, they should administer the next treatment as soon as they recognized that they have missed a dose, and then resume the originally scheduled monthly application routine as rapidly as possible to reinstitute the steady-state protection.

### Conclusion

A single topical application of 10% imidacloprid + 2.5% moxidectin topical solution (Advantage Multi® for Dogs) administered per label 30 days prior to challenge was 100% efficacious in preventing the development of third-stage larvae of the Missouri isolate of *D. immitis* in dogs. A monthly regimen of 10% imidacloprid + 2.5% moxidectin topical solution appears to provide protection against the development of heartworms throughout the entire month between applications and not just for the 30 days prior and one to several days following product administration. The American Heartworm Society has suggested that poor owner compliance in adhering to this 30-day dosing interval is the leading cause for lack of efficacy of heartworm preventives [12]. Due to the shorter half-lives and lack of any evidence that other monthly heartworm preventives develop a protective steady state, strict adherence to their 30-day dosing interval is required in order to ensure efficacy as per label instructions. Based on this study and prior research on the steady-state phenomenon that occurs after the regular monthly application of Advantage Multi® for Dogs [4], the endpoint of protection is longer than 30 days and indistinct; if a dose is missed by a few days, there is still sufficient product in the dog to be protective. Therefore, because owners sometimes inadvertently forget or cannot apply prevention on the same day every month, Advantage Multi® for Dogs should provide more peace of mind to owners and veterinarians. The monthly use of heartworm preventive products, however, such as Advantage Multi for Dogs®, should always be recommended.

### Abbreviations

IAUC: Institutional Animal Care and Use Committee; IVP: Investigational Veterinary Product; SD: Study day

### Acknowledgements

The authors would like to thank Dr. Byron Blagburn of Auburn University, Auburn, AL, for supplying the Missouri isolate used in the study, and Ms. Joy Bowles, Ms. Jamie Butler, and Dr. Lindsay Starkey of Auburn University and Jeff Gruntmeir of the University of Florida for assisting with the inoculation of the dogs with larvae.

### Funding

The study was funded by Bayer Animal Health. The article’s publication fee was funded by the American Heartworm Society.
Availability of data and materials
Data leading to the presented conclusions are available in the article.

About this supplement
This article has been published as part of Parasites and Vectors Volume 10: Supplement 2, 2017: Proceedings of the 15th American Heartworm Society Triennial Symposium 2016. The full contents of the supplement are available online at https://parasitesandvectors.biomedcentral.com/articles/supplements/volume-10-supplement-2.

Authors’ contributions
DDB served as primary investigator on the study and oversaw all major study related events including infection, treatment, and necropsy. The study was designed and implemented through the direction of the Bayer team that consisted of CO, DJK, JAH, and SDC. TLS was responsible for the statistical analysis. All the authors read and approved the manuscript.

Ethics approval
The study was approved by the facility Institutional Animal Care and Use Committee (IACUC) with the concurrence of Bayer Animal Health and followed the guidelines of Guidance for Industry 85 from the Center for Veterinary Medicine of the US Food and Drug Administration.

Consent for publication
Not applicable.

Competing interests
In the past 5 years, DDB has received reimbursement, speaking fees, or research support from Bayer Animal Health, manufacturer of the heartworm preventive used in this research. All other authors are employees of Bayer Animal Health.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Liberty Research Inc, Waverly, NY, USA. 2Bayer Animal Health, Shawnee, KS, USA. 3Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, 930 Campus Road, Ithaca, NY 14853, USA.

Published: 9 November 2017

References
1. Samson-Himmelstjerna G, Von Epe C, Schimmel A, Heine J. Larvicidal and persistent efficacy of an imidacloprid and moxidectin topical formulation against endoparasites in cats and dogs. Parasitol Res. 2003;90:S114–5.
2. Cruthers LR, Arther RG, Base CL, Charels SD, Hostetler JA, Settje TL. New developments in parasite prevention. In: Bayer Selected Proceedings, NAVC conference; 2008. p. 15–20.
3. Little SE, Hostetler JA, Thomas JE, Bailey KL, Barrett A, Gunttmir K, et al. Moxidectin steady state prior to inoculation protects cats from subsequent, repeated infection with Dirofilaria immitis. Parasit Vectors. 2015;8:107.
4. Bowman DD, Grazette AR, Basel C, Wang Y, Hostetler JA. Protection of dogs against canine heartworm infection 28 days after four monthly treatments with Advantage Multi® for dogs. Parasit Vectors. 2016;9:12.
5. US Food and Drug Administration, Center for Veterinary Medicine VICH GL9. Guidance for Industry Good Clinical Practice, VICH GL9 Final Guidance. 2001 (http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052417.pdf). Accessed May 13, 2017.
6. Al-Azzam SI, Fleckenstein L, Cheng KJ, Drimanski MT, McCall JW. Comparison of the pharmacokinetics of moxidectin and ivermectin after oral administration to beagle dogs. Biopharm Drug Dispos. 2007;28:431.
7. Dauro CP, Cheung EN, Jeffcoat AR, Skelly BJ. Bioavailability of ivermectin administered orally to dogs. Vet Res Commun. 1992;16:125–30.
8. Jung M, Saito A, Buescher G, Maurer M, Graf JF. Chemistry, pharmacology and safety of the macrocyclic lactones: milbemycin oxime. In: Vercruysse J, Rew RS, editors. Macrocylic lactones in Antiparasitic therapy. Wallingford: CAB International; 2002. p. 51–74.
9. US Food and Drug Administration, Animal & Veterinary, NADA 141-251 ADVANTAGE MULTI for Dogs Imidacloprid + Moxidectin - original approval. December 2006. https://www.fda.gov/downloads/AnimalVeterinary/ApprovedAnimalDrugProducts/FOIADrugSummaries/UCM051438.pdf. Accessed May 13, 2017.
10. Blagburn BL, Dillon AR, Arther RG, Butler JM, Newton JC. Comparative efficacy of four commercially available heartworm preventive products against the MP3 laboratory strain of Dirofilaria immitis. Vet Parasitol. 2011;176:89–94.
11. European Medicines Agency (EMA), Committee for Medicinal Products for Veterinary Use (CVMP) 2014. Assessment Report for NEXGARD SPECTRA (EMEA/V/C/003842/0000). http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/veterinary/003842/WC500181963.pdf. Accessed May 13, 2017.
12. American Heartworm Society. Current canine guidelines for the prevention, diagnosis, and Management of Heartworm (Dirofilaria immitis) infection in dogs. Wilmington: American Heartworm Society; 2014. p. 20.