AMERICAN HEARTWORM SOCIETY™
EST. 1974

BULLETIN

LEADING THE FIGHT AGAINST HEARTWORM DISEASE

SEPTEMBER
2017
VOLUME 44 No. 3

HEARTWORM Q&A

Heartworm History: In What Year Was Heartworm First Treated?

Questions from members, practitioners, technicians, and the general public are often submitted to the American Heartworm Society (AHS) via our website. Two of our AHS Board members, Dr. John W.McCall and Dr. Tom Nelson, provided the resources to answer this question: In What Year Was Heartworm First Treated?

The first efforts to treat canine heartworm disease date back to the 1920s. Dr. Nelson referenced a review article by Dr. Raffaele Roncalli, "Tracing the History of Heartworms: A 400 Year Perspective," published in the 1998 AHS Symposium Proceedings.[1] Dr. Roncalli wrote, "The first trial to assess the efficacy of a microfilaricide (natrium antimonyl tartrate) was conducted some 70 years ago (1927) in Japan by S. Itagaki and R. Makino.[2] Fuadin (stibophen), a trivalent antimony compound, was tested, intravenously, as a microfilaricide by Popescu in 1933 in Romania and by W.H. Wright and P.C. Underwood in 1934 in the USA. In 1949, I.C. Mark evaluated its use intraperitoneally."

Continues on page 7

INSIDE THIS ISSUE

OUR GENEROUS SPONSORS

PLATINUM LEVEL

Bayer HealthCare Animal Health

Boehringer Ingelheim

Ceva

Elanco

Virbac Animal Health

zoetis

SILVER LEVEL

IDEXX Laboratories

MERCK Animal Health

AMERICAN HEARTWORM SOCIETY™ EST. 1974

PO Box 8266
Wilmington, DE 19803-8266
info@heartwormsociety.org
www.heartwormsociety.org

Mission Statement

The mission of the American Heartworm Society is to lead the veterinary profession and the public in the understanding of heartworm disease.

Heartworm Vision

The vision of the American Heartworm Society is a world without heartworm.

HEARTWORM UNIVERSITY AMERICAN HEARTWORM SOCIETY™

2016 TRIENNIAL SYMPOSIUM AMERICAN HEARTWORM SOCIETY™

Help us in our mission to lead the veterinary profession and the public in understanding heartworm disease.

We have committees that could use your expertise and enthusiasm.

Contact us at **info@heartwormsociety.org**

2017 EXECUTIVE BOARD

Officers

Dr. Christopher J. Rehm, Sr.
President
Rehm Animal Clinic, P.C.
Mobile, AL

Dr. Tony Rumschlag
Vice President
Elanco
Greenfield, IN

Dr. Patricia Payne
Editor
Emeritus Faculty
Kansas State University
Manhattan, KS

Dr. Stephen Jones
Past-President
Lakeside Animal Hospital
Moncks Corner, SC

Dr. Bianca Zaffarano
Secretary/Treasurer
Iowa State University
Ames, Iowa

Board Members

Dr. Elizabeth Clyde
Clyde's Animal Clinic
Mattoon, IL

Dr. Christopher Duke
Bienville Animal Medical Center
Ocean Springs, MS

Dr. Tom Nelson
Animal Medical Center
Anniston AL

Dr. Brian DiGangi
Senior Director of
Shelter Medicine
ASPCA
Gainesville, FL

Dr. Andrew Moorhead
University of Georgia
Athens, GA

Dr. Jenni Rizzo
Mission Pet Emergency
San Antonio, TX

Ex-Officio — Non-Voting

Dr. Chris Adolph
Zoetis
Tulsa, OK

Dr. Doug Carithers
Symposium
Program Chair
Boehringer
Ingelheim
Duluth, GA

Dr. John McCall
Associate Editor
Professor Emeritus
University of GA
Athens, GA

Dr. Robert Zolynas
Bayer HealthCare
Kansas City, MO

Dr. Marisa Ames
Colorado State
University
Fort Collins, CO

Dr. Elizabeth Hodgkins
Ceva Animal Health
Lenexa, KS

Dr. Craig Parks
Virbac
Fort Worth, TX

Association Management Team

Dr. Kathy Gloyd
ELEVATE DVM
Wilmington, DE
kathy.gloyd@heartwormsociety.org

Bonnie Gamble
ELEVATE DVM
bonnie.gamble@
heartwormsociety.org

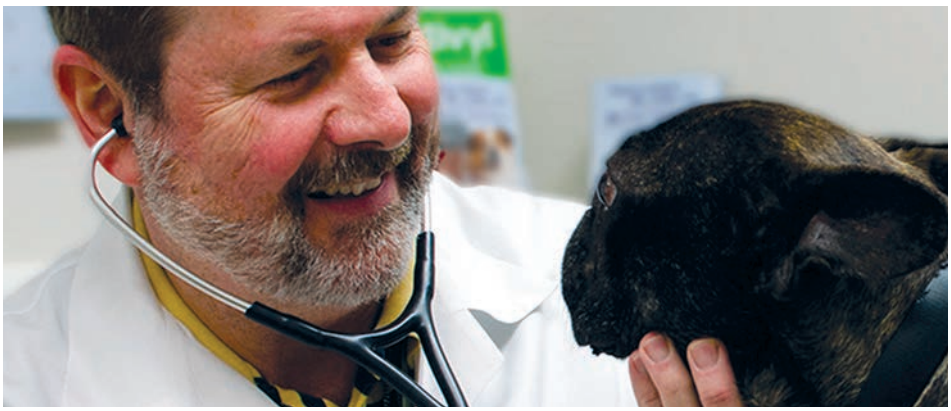
Sonya Hennessy
(Heartworm University)
ELEVATE DVM
sonya.hennessy@elevatedvm.com

Robin Hipple
(Bulletin)
ELEVATE DVM
robin.hipple@elevatedvm.com

Lisa Scott
ELEVATE DVM
lisa.scott@heartwormsociety.org

PR CONTACT
Sue O'Brien
Sue K. O'Brien Communications
obriensuek@gmail.com

Time Flies, Science Does Not



Christopher J. Rehm, Sr., DVM, President

It seems like just yesterday we were gearing up for the 15th AHS Triennial Symposium, held last September in New Orleans. How time flies! We are very pleased to report that the 2016 Symposium broke the attendance record set in 2013, received excellent reviews from attendees, and is on the way to having a record number of articles published in the Symposium proceedings in *Parasites & Vectors*!

While time is flying by, science is trying to keep up with new topics, new information, and new developments in the very interesting and complicated parasite we love to study. Over the years I have heard many AHS Board members, especially past presidents, say "I just did not know how much I did not know" about heartworm disease and infection. I thought I had a pretty good grasp on the subject until I sat in on my first board meeting in 2010. My head was spinning with all the information, questions, studies, and efforts that the AHS works on so diligently and so tirelessly behind the scenes.

SCIENCE AND RESEARCH BACK UP EVERYTHING WE DO AT AHS

As an AHS Board member and now your President, I attend many meetings and get many questions. We also receive many questions on the AHS website (heartwormsociety.org) (see page 1 of this issue for the AHS response to a recent question on the history of heartworm treatment). Practitioners want answers and guidance, as do pet owners, researchers, and teachers of our future veterinarians. We are working hard to get those answers, but science and research take time. We have a great team of board members who communicate regularly to be sure we get good, solid, accurate information out to our practitioners, techs, and the public.

For example, I am very proud of the fact that we reference all the information in our Guidelines. We also wait until new research is published in peer-reviewed journals before making changes in our Guidelines or recommendations. There are many nuances in the art of treating infected pets for heartworm. We hear the cries to shorten or make our treatment protocol easier. We simply will not change what has worked so well until we have science to back up any changes the board deems necessary.

TRANSLATING RESEARCH INTO PRACTICE

Research has given us safe and effective products to prevent and treat heartworm disease and infection. Sometimes I bemoan the fact that many have been spoiled by how well these products work. I see a good bit of noncompliance in my practice where, thanks to neighbors and good community compliance, a client gets lucky and their pet is not exposed during those lapses in prevention dosing. We still have to make the plea to our clients on behalf of our patients to protect their pets all year long, with either every-30-day dosing or every-6-month injections. We must get our clinic teams, our colleagues, our teachers in colleges of veterinary medicine – in all areas of the country – and the public to buy in to every pet, every visit, every time, and no pet left behind in the fight against this deadly parasite. The pathology is too devastating and often lingers for years after treatment, even until the death of the pet.

EDUCATION AND OUTREACH ARE CRITICAL TO ACHIEVING OUR MISSION

If this disease were truly understood, then everyone would do everything in their power to make sure their beloved pet or patient was never exposed and if exposed, would have prevention on board to protect the pet from developing heartworm disease. It is a "no-brainer."

Our mission at AHS is to lead the veterinary profession and the public in the understanding of heartworm disease. Education is critical. I am encouraged in the growing interest in heartworm across the country. Thanks to the efforts of AHS Board member Dr. Jenni Rizzo, we are actively increasing veterinary student participation in AHS (for more on this, see page 21). These are the future researchers and practitioners who will bring us new breakthroughs in heartworm prevention, diagnostics, and treatment.

I started my letter talking about the great AHS Symposia of the past. Time flies—in two short years we will be in New Orleans again, at the same world-class venue, the Ritz Carlton. We can only imagine the findings of scientific research now under way that will be reported there. I hope you will put the 16th AHS Triennial Symposium on your calendar—September 8–11, 2019—and plan to join us.

– Christopher J. Rehm, Sr., DVM

AMERICAN HEARTWORM SOCIETY™
EST. 1974

"Like" us on Facebook at
facebook.com/heartwormsociety

Follow us on Twitter at
twitter.com/AHS_Think12

Check out our educational and client education videos on
youtube.com/user/americanheartworm

For more on Fuadin (stibophen), Dr. McCall cited a report by Dr. Ron Jackson, one of the founders of the American Heartworm Society and its first president, "The History of Heartworm," published in the Proceedings of the California Heartworm Symposium in January 1989 as a special edition of the *California Veterinarian*.[3] Dr. Jackson wrote that from 1933 to 1945, "Stibophen (Fuadin) was used to treat heartworm. This antimonial compound was the only available treatment at that time. It eliminated microfilaria and because they did not reappear, it was thought that it also destroyed adult worms. Later it was found that adult worms were sterilized, not destroyed. This treatment was used by the author in the early 1940s and the drug was found to be toxic."

Dr. Nelson also cited a 1945 National Research Council Publication: "A Report on the Medicinal Treatment of *Filariasis Bancrofti* Related to Experiments on Animals."[4] The article discusses the compounds used to try to treat *Dirofilaria immitis* in dogs as a model to find a treatment for human filariasis. Some early compounds tried were formalin and atoxyl, an organoarsenic compound used to treat trypanosomiasis in the early 1900s.

Perhaps a more useful question, however, is, "In what year was the first dog/animal *successfully* treated for heartworms?

Adult heartworms were first successfully treated in dogs in 1947 using the arsenical compound thiacetarsamide. According to Dr. Jackson's report, "In 1945, thiacetarsamide was developed by Dr. Gilbert Otto and Dr. Thomas Martin. At that time, Dr. Otto, a parasitologist at Johns Hopkins University, was employed by the Navy to find a treatment for human filariasis (*Wuchereria bancrofti*) that was common in the Pacific islands. In the process he discovered the adulticidal effects of thiacetarsamide on adult *D. immitis* in 13 infected dogs that were provided by the author [Dr. Jackson] from St. Augustine, Florida. The drug was marketed commercially in 1949. The dose schedule was 0.1 mL/lb daily for 15 days, and this dose was used successfully for about the next 10 years."[3]

This photomicrograph of a mosquito proboscis ejecting heartworms, taken by Dr. John W. McCall, appeared on the cover of the January 1989 special issue of the *California Veterinarian*, which included the proceedings of the California Heartworm Symposium.

In 1957–1956, "After experimenting with various dose schedules of thiacetarsamide, Dr. S. Kume and later Major Robert Bailey, a US Air Force veterinarian working in Japan, presented evidence that 0.2 mL/lb thiacetarsamide daily for two days would eliminate adult heartworms. This schedule was then adopted by most practitioners in the US, although there was higher incidence of toxic drug reactions with this dose and also some fatalities."[3]

The use of dithiazanine iodide (Dizan) as a microfilaricide was first reported in 1959 by Dr. James Yarborough and colleagues. Its microfilaricidal activity was discovered serendipitously while evaluating its efficacy for the treatment of hookworms and other nematodes. Up until this point, the only microfilarial agent was Fuadin, which was not widely used due to its toxicity.

Finally, in 1989, "R.B. Atwell and A.C.E. Searle, M.T. Dzimianski and colleagues, and other investigators reported on the activity of melarsomine dihydrochloride, a trivalent arsenical, used intramuscularly against immature and adult heartworms. Melarsomine dihydrochloride was introduced into the U.S. market in 1995, after being licensed in Australia, Italy, France, and Japan."[1]

Melarsomine remains the only adulticidal drug approved by the FDA today for treatment of canine heartworm disease.

For more information on recommend treatment protocols, please refer to the AHS Canine Guidelines available on the AHS website, **heartwormsociety.org**.

If you have a question about heartworm prevention, diagnosis, or management, please check our **Frequently Asked Questions** on the AHS website, or contact us at **info@heartwormsociety.org**.

References

1. Roncalli R. Tracing the history of heartworms: A 400 year perspective. In *Recent Advances in Heartworm Disease: AHS Symposium '98*. American Heartworm Society, 1998.
2. Itagaki S, Makino R. Studies on the treatment of microfilaria immitis infection by intravenous injection of natrium antimonyl tartarate. *J Jap Soc Vet Sci*. 1927;6:15-23.
3. Jackson RF. The history of heartworm. In *Proceedings of the California Heartworm Symposium. California Veterinarian*. January 1989, special issue.
4. National Research Council. A Report on the Medicinal Treatment of *Filariasis Bancrofti* Related to Experiments on Animals. 1945.

ABSTRACTS FROM THE LITERATURE

Journals piling up and no time to read? Get caught up on the latest research findings with these abstracts from recently published heartworm-related studies.

Pulmonary hypertension in dogs with heartworm before and after the adulticide protocol recommended by the American Heartworm Society

B. Serrano-Parreño,[1] E. Carretón,[1,*] A. Caro-Vadillo,[2] S. Falcón-Cordón,[1] Y. Falcón-Cordón,[1] and J.A. Montoya-Alonso[1]

1 Internal Medicine, Faculty of Veterinary Medicine, Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain
2 Department of Animal Medicine and Surgery, Veterinary Faculty, Complutense University of Madrid, Madrid, Spain
* Corresponding author: elena.carreton@ulpgc.es

From *Veterinary Parasitology* 2017;236:34-37.

Pulmonary hypertension (pH) is a frequent and severe phenomenon in heartworm disease (*Dirofilaria immitis*). There is a lack of studies assessing the evolution of the proliferative endarteritis and pH caused by *D. immitis* after the death of the parasites, so this study evaluated the influence that the elimination of the worms exerts over the pulmonary pressure and therefore evolution of the endarteritis, through the evaluation of the Right Pulmonary Artery Distensibility (RPAD) Index and other echocardiographic measurements in 2D mode, M-mode and Doppler echocardiography in 34 dogs naturally infected by *D. immitis* on day 0, and one month after the last adulticide dose (day 120). pH, based on the determination of the RPAD Index, was present in 68% of the dogs (n = 23) on day 0 and on day 120. No significant differences were observed between the RPAD Index between the two measurements, and only significant differences were found in pulmonary deceleration time, ejection time, and left ventricular internal diameter in telediastole when measurements from day 0 and day 120 were compared. There was not any worsening in the development of pH after the elimination of the parasites, independently of the parasite burden. During the adulticide treatment, the death of the worms causes thromboembolism and tends to worsen the vascular damage and presence of pH. It seems that following the adulticide protocol recommended by the American Heartworm Society with the previous elimination of *Wolbachia* and reduction of microfilariae followed by the stepped death of the worms did not cause a significant aggravation of the pulmonary damage of the treated dogs. Neither is present any significant improvement in the RPAD Index on day 120; probably, more time is needed before appreciating some positive changes after the elimination of the worms and *Wolbachia* from the vasculature and further studies are necessary.

KEYWORDS: Heartworm, *Dirofilaria immitis*, Echocardiography, Pulmonary hypertension, Pulmonary artery, Endarteritis

Filarioid infections in wild carnivores: a multispecies survey in Romania

A.M. Ionic,[1,*] I.A. Matei,[2] G. D'Amico,[2] J. Ababii,[2] A.A. Daskalaki,[2] A.D. Sándor,[2] D.V. Enache,[3] C.M. Gherman,[2] and A.D. Mihalca[2]

1 Department of Parasitology and Parasitic Diseases, Faculty ofVeterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372, Cluj-Napoca, Romania. ionica.angela@usamvcluj.ro.
2 Department of Parasitology and Parasitic Diseases, Faculty ofVeterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăştur 3-5, 400372, Cluj-Napoca, Romania.
3 Department of Engineering and Food and Tourism Management, Faculty of Food and Tourism, Transilvania University, Castelului Street, 500014, Braşov, Romania.
* Corresponding author: ionica.angela@usamvcluj.ro.

From *Parasites & Vectors* 2017;July 13;10(1):332. doi: 10.1186/s13071-017-2269-3.

BACKGROUND: Filarioids are vector-borne parasitic nematodes of vertebrates. In Europe, eight species of filarioids, including zoonotic species, have been reported mainly in domestic dogs, and occasionally in wild carnivores. In Romania, infections with *Dirofilaria* spp. and *Acanthocheilonema reconditum* are endemic in domestic dogs. Despite the abundant populations of wild carnivores in the country, their role in the epidemiology of filarioid parasites remains largely unknown. The aim of the present study was to assess the host range, prevalence and distribution of filarioid infections in wild carnivores present in Romania.

METHODS: Between May 2014 and February 2016, 432 spleen samples originating from 14 species of wild carnivores have been tested for the presence of DNA of three species of filarioids (*D. immitis, D. repens* and *A. reconditum*).

RESULTS: Overall 14 samples (3.24%) were molecularly positive. The most prevalent species was *D. immitis* (1.62%), accounting for 50% ($n = 7$) of the positive animals. The prevalence of *D. repens* was 1.39%, while that of *A. reconditum* was 0.23%. No co-infections were detected. *Dirofilaria immitis* DNA was detected in five golden jackals, *Canis aureus* (7.58%), one red fox, *Vulpes vulpes* (0.33%), and one wildcat, *Felis silvestris* (10%). The presence of *D. repens* DNA was detected in two red foxes (0.66%), two golden jackals (3.03%), one grey wolf (7.14%), and one least weasel, *Mustela nivalis* (33.33%). *Acanthocheilonema reconditum* DNA was found only in one red fox (0.33%).

CONCLUSION: The present study provides molecular evidence of filarial infections in wild carnivore species in Romania, suggesting their potential epidemiological role and reports a new host species for *D. repens*.

KEYWORDS: *Acanthocheilonema reconditum, Dirofilaria* spp., Infection, Romania, Wild carnivores

Canine infection with *Borrelia burgdorferi, Dirofilaria immitis, Anaplasma* spp. and *Ehrlichia* spp. in Canada, 2013–2014

B.H. Herring,[1,*] A.S. Peregrine,[2] J. Goring,[3] M.J. Beall,[3] and S.E. Little[4]

1 Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA.
2 Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.
3 IDEXX Laboratories, Inc, Westbrook, ME, USA.
4 Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater, OK, USA.
* Corresponding author: brian.h.herrin@okstate.edu.

From Parasites & Vectors 2017; May 19;10(1):244. doi: 10.1186/s13071-017-2184-7.

BACKGROUND: Canine test results generated by veterinarians throughout Canada from 2013–2014 were evaluated to assess the geographical distribution of canine infection with *Borrelia burgdorferi, Dirofilaria immitis, Ehrlichia* spp., and *Anaplasma* spp.

METHODS: The percent positive test results of 115,636 SNAP® 4Dx® Plus tests from dogs tested were collated by province and municipality to determine the distribution of these vector-borne infections in Canada.

RESULTS: A total of 2,844/115,636 (2.5%) dogs tested positive for antibody to *B. burgdorferi*. In contrast, positive test results for *D. immitis* antigen and antibodies to *Ehrlichia* spp. and *Anaplasma* spp. were low, with less than 0.5% of dogs testing positive for any one of these three agents nationwide. Provincial seroprevalence for antibodies to *B. burgdorferi* ranged from 0.5% (Saskatchewan) to 15.7% (Nova Scotia); the areas of highest percent positive test results were in proximity to regions in the USA considered endemic for Lyme borreliosis, including Nova Scotia (15.7%) and Eastern Ontario (5.1%). These high endemic foci, which had significantly higher percent positive test results than the rest of the nation ($P < 0.0001$), were surrounded by areas of moderate to low seroprevalence in New Brunswick (3.7%), Quebec (2.8%), and the rest of Ontario (0.9%), as well as northward and westward through Manitoba (2.4%) and Saskatchewan (0.5%). Insufficient results were available from the westernmost provinces, including Alberta and British Columbia, to allow analysis.

Research Update conitnued from Page 9

CONCLUSION: Increased surveillance of these vector-borne disease agents, especially *B. burgdorferi*, is important as climate, vector range, and habitat continue to change throughout Canada. Using dogs as sentinels for these pathogens can aid in recognition of the public and veterinary health threat that each pose.

KEYWORDS: *Anaplasma, Borrelia burgdorferi*, Canada, Canine, *Dirofilaria immitis*, Ehrlichia

Heat pretreatment of canine samples to evaluate efficacy of imidacloprid + moxidectin and doxycycline in heartworm treatment

A.J.R. Bendas,[1,*] F. Mendes-de-Almeida,[2] C. Von Simson,[3] and N. Labarthe,[2,4]

1 Programa de Pós-Graduação em Medicina Veterinária, Faculdade de Veterinária Universidade Federal Fluminense, R. Vital Brazil Filho, 64, Santa Rosa, Niterói-RJ, 24230-340, Brazil. 2 Programa de Pós-Graduação em Medicina Veterinária, Faculdade de Veterinária Universidade Federal Fluminense, R. Vital Brazil Filho, 64, Santa Rosa, Niterói-RJ, 24230-340, Brazil.
3 Veterinary Services, Virbac US P.O. box 162059, Ft Worth, TX, 76161, USA.
4 Fundação Oswaldo Cruz, Av. Brasil, 4365, Rio de Janeiro, RJ, 21040-900, Brazil.
* Corresponding author: alexandrebendas@gmail.com.

From *Parasites & Vectors* 2017 May 19;10(1):246. doi: 10.1186/s13071-017-2189-2.

BACKGROUND: Considering the recent information on the increase of *Dirofilaria immitis* antigen detection by rapid assays in canine blood samples after heat treatment, the proposal that immune complexes block *D. immitis* antigen detection and that macrocyclic lactone + doxycycline (alternative protocol) might lead to increased production of those immune complexes, resulting in the erroneous diagnosis of adult worm elimination, and that there is no recommended adulticide marketed in Brazil, a study was performed to evaluate the interference of moxidectin + doxycycline (moxi-doxy) on diagnostic procedures when heartworm positive dogs are treated with this alternative protocol. Twenty-two naturally infected pet dogs were treated monthly with topical 10% imidacloprid + 2.5% moxidectin and with oral doxycycline (10 mg/kg BID/30 days) (moxi-doxy). All the dogs had their microfilaremia level determined prior to the first day of treatment, and were tested every 6 months for microfilariae (Mf) detection prior to heating, and for antigen detection prior to and after heating, the sample.

RESULTS: The results indicate that the treatment protocol can eliminate adult heartworms as early as 6 months after the first dose, especially in low microfilaremic dogs (< 300 Mf/ml). In this study, all dogs were free of heartworm antigen after 18–24 months of treatment. In a comparison of pre-heated samples and non-heated samples, sample pre-heating increased antigen detection sensitivity, and non-heated samples tended to be antigen-negative earlier than the pre-heated samples, especially when dogs had low microfilaremia levels. These discrepancies were not present in a subsequent sample of the same dog 6 months later.

CONCLUSIONS: Two negative antigen test results 6 months apart can be recommended as the criterion to consider when a dog has been cleared of infection. The initial microfilaremia level of a dog can be used to estimate the necessary time frame to end the treatment period.

KEYWORDS: Adulticide treatment, Antigen detection, *Dirofilaria immitis*, Heartworm

Periodicity of *Dirofilaria immitis* in Long-term Infections

Christopher C. Evans,[1,*] Erica J. Burkman,[1] Michael T. Dzimianski,[1] Andrew R. Moorhead,[1] Molly D. Savadelis,[1] Carsten Angenendt,[2] Sven Zymny,[2] and Daniel Kulke[2]

1 Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA
2 Bayer Animal Health GmbH, Drug Discovery, Parasiticides, Filaricides Research, 51368 Leverkusen, Germany
* Corresponding author: ccevans@uga.edu

From *Parasitology Research* 2017;116:S75–S80 DOI 10.1007/s00436-017-5493-z

The concentration of *Dirofilaria immitis* microfilariae in peripheral blood samples was measured in four experimentally infected dogs. Samples were collected at hourly intervals from 6.30h to 17.30h from all dogs at 11, 22, and 27 months post infection, and at 39 months post infection for two dogs only. Microfilarial periodicity follows the form of a simple harmonic wave over a 24-hour period, and concentration data was fit to sine wave for each sample date to characterize changes in periodicity over time. We found the periodicity index (i.e., wave amplitude) to decrease with time ($p = 0.016$, $R2 = 0.97$) dropping from 74.57

Continues on page 12

Research Update conitnued from Page 10

(95% CI, 63.79 to 85.34) at 11 months post-infection to 5.55 (95% CI, 0 to 14.82) at 39 months post infection. The time of peak microfilaremia was calculated to be 17.36 h (95% CI, 17.01h to 18.08 h) at 11 months post infection and did not change significantly with time ($p = 0.17$, $R2 = 0.70$). No significant trend was observed in total microfilarial count for individual dogs ($p > 0.10$). The data presented here indicate a gradual but significant loss of periodicity over the two-year study period despite maintenance of overall microfilarial levels.

KEYWORDS: *Dirofilaria immitis*, Canine, Periodicity, Microfilariae

Temporal Pattern of Circulating Antigens and Antibody Responses in Cats Experimentally Infected with *Dirofilaria immitis*

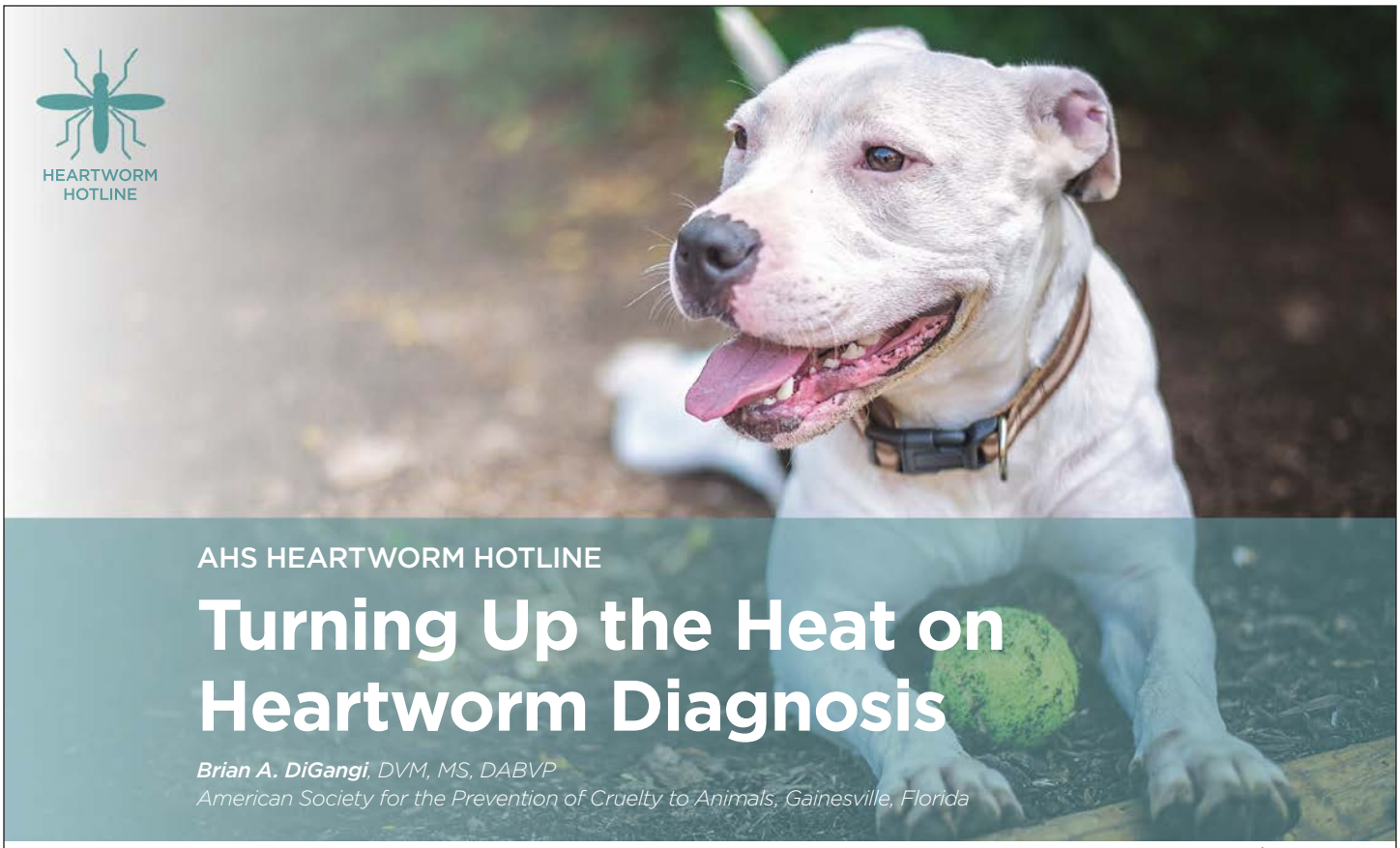
Kris Curtis,[1] Tom O'Connor,[1] Leif Lorentzen,[1] John McCall,[2] Michael Tillson,[3] Ray Dillon,[3] and Ramaswamy Chandrashekar,[1,*]

1 IDEXX Laboratories, Inc., Westbrook, ME 04092, USA
2 University of Georgia, Athens, GA 30602, USA
3 Auburn University, Auburn, AL 36849, USA
* Corresponding author: Chandra@idexx.com

Heartworm disease in cats has been attributed to immature adult heartworms reaching the pulmonary arteries approximately 2.5 to 4 months post infection and the presence of mature adult worms in the cardiopulmonary system approximately 6 months after infection. The arrival and death of immature heartworms causes significant lung pathology, a condition referred to as Heartworm-Associated Respiratory Disease (HARD). To evaluate the humoral immune response to *Dirofilaria immitis*, 12 cats were each infected by subcutaneous injection of 100 third-stage larvae. Postinfection serum samples were collected weekly for 4 weeks, every other week for the following 4 weeks, then monthly to day 270 for evaluation of antibodies to *D. immitis* recombinant antigens

HWAg-1 and HWAg-2 and circulating heartworm antigen (HWAg) using the PetChek® HTWM PF Test Kit (IDEXX Laboratories). Necropsies were performed 278 to 299 days post infection for collection of adult worms. Eleven cats were HWAg-1 antibody-positive 68 days post infection, and the remaining cat became positive by day 140. Eleven cats were positive for HWAg-2 antibody 42 to 84 days post infection; all 11 remained HWAg-2 antibody positive through day 270. Circulating HWAg was detected in 10 cats, two by day 140 and eight others by day 168. The two antigen-negative cats had no adult worms at necropsy. This study demonstrates that decomposition of immature adult heartworms can result in detectible levels of circulating antigen prior to sexual maturation. In this experimental model, *D. immitis antigen* was detectable in cats at time points (days 140 and 168) associated with HARD from dying immature adult heartworms.

KEYWORDS: Heartworm, Feline, Heartworm-Associated Respiratory Disease (HARD), Immune response, Circulating antigens



HEARTWORM HOTLINE

AHS HEARTWORM HOTLINE

Turning Up the Heat on Heartworm Diagnosis

***Brian A. DiGangi**, DVM, MS, DABVP*
American Society for the Prevention of Cruelty to Animals, Gainesville, Florida

shutterstock.com/atiger

The **Heartwork Hotline** column is presented in partnership between ***Today's Veterinary Practice*** and the **American Heartworm Society** (**heartwormsociety.org**). The goal of the column is to communicate practical and timely information on prevention, diagnosis, and treatment of heartworm disease, as well as highlight current topics related to heartworm research and findings in veterinary medicine.

AMERICAN HEARTWORM SOCIETY™ EST. 1974

Antigen testing for *Dirofilaria immitis* has been a foundational component of model preventive veterinary care for many years, particularly for dogs. For privately owned pets, the results of such testing guide prevention strategies and, in the event of a positive result, treatment for heartworm disease. In shelter populations, the results are often a key determining factor in the management of a dog throughout its stay in the shelter system, including its likelihood of a live release.

Although the accuracy of commercially available *D immitis* antigen test kits has been widely studied, recent reports have sparked renewed interest in the effect of antigen–antibody immune complexes on test results.[1–6] Such complexes can interfere with antigen detection, resulting in "no antigen detected" (NAD) test results in infected animals, and should be considered when NAD test results conflict with clinical expectations.

WHAT ARE IMMUNE COMPLEXES?

Immune complexes represent soluble antigen bound to endogenous antibody, forming an insoluble unit that remains in circulation. Such complexes are a normal component of a functioning immune system and are cleared by phagocytosis when the balance of antigen to antibody in circulation is maintained. Excess immune complexes in circulation can result in tissue deposition, leading to local inflammatory responses and a variety of autoimmune diseases. Vasculitis, glomerulonephritis, pneumonitis, and arthritis are common sequelae of immune complex deposition in the respective affected organ system. In the case of diagnostic testing methods that rely on soluble antigen for detection (eg, enzyme-linked immunosorbent assay, lateral flow assays), antigen bound in an immune complex may not be available for detection, leading to an NAD result despite the presence of antigen in the test sample.

A variety of techniques can be used to dissociate circulating immune complexes in a diagnostic sample.

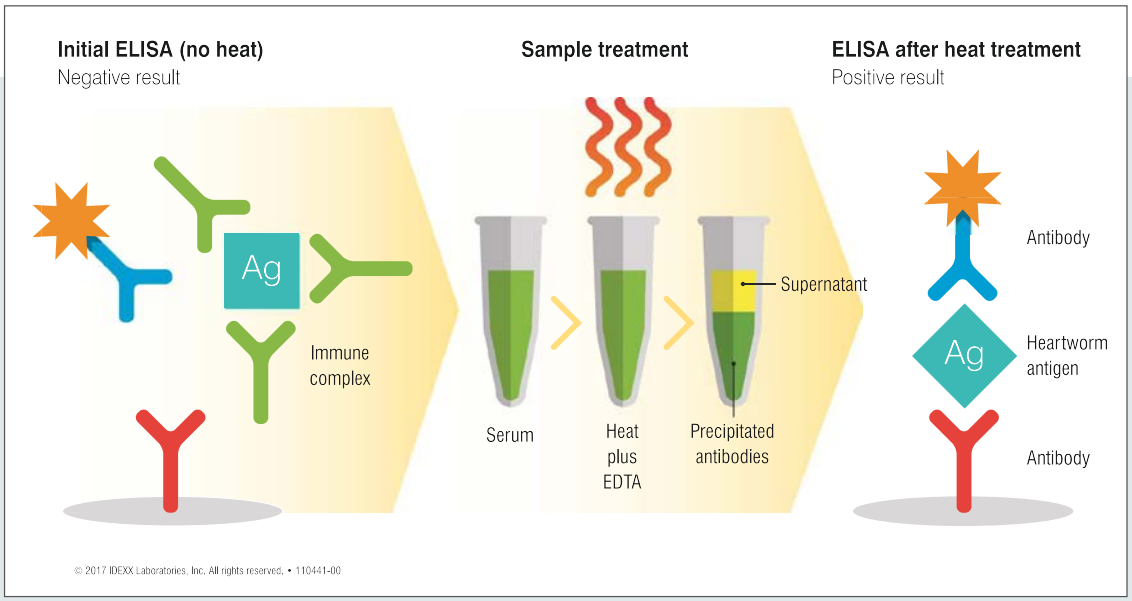


FIGURE 1. Immune complex dissociation with sample pretreatment. Figure used with permission from IDEXX Laboratories, Inc. Ag, antigen; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay.

Such techniques rely on denaturing proteins within the complex, allowing for precipitation of antibodies and subsequent freeing of the antigen (**Figure 1**). In laboratory settings, proteolytic enzymes (eg, pepsin), acid treatment (eg, ethylenediaminetetraacetic acid, citric acid), heat (104°C for 10 minutes), or a combination of these methods is frequently used for immune complex dissociation (ICD).

Heat pretreatment (HPT) of serum samples was standard practice in veterinary diagnostic laboratories through the mid-1990s and is still available upon request. However, the demand for simple, cost-effective, commercially available, point-of-care test kits led to its decreased use.

HOW DOES HEAT PRETREATMENT AFFECT DIAGNOSIS OF HEARTWORM INFECTION?

In recent years, the effect of ICD in the form of HPT has been studied in diagnostic samples from cats and dogs.[1–6] Such reports have demonstrated substantial increases in antigen detection in both species, resulting in greater diagnostic sensitivity (**Table 1**); however, the antigen detected cannot be identified as coming from living or dead heartworms. It follows that in dogs with heartworm disease that have received adulticidal therapy, a positive result on an antigen test with ICD does not indicate that the therapy was unsuccessful.

TABLE 1 Effect of Heat Pretreatment on Antigen Detection

REFERENCE	POPULATION	STANDARD	HEAT
Ciucă et al 2016[1]	194 Romanian stray dogs	8.2%	27%
DiGangi et al 2016[2]	616 shelter dogs in United States	7.3%	12.3%
Drake et al 2015[3]	15 owned dogs	0%*	53%
Gruntmeir et al 2015[4]	34 owned dogs	0%*	67.4%
Little et al 2014[5]	6 experimentally infected cats	17%	83%
Little et al 2014[6]	220 shelter cats in United States	0.45%	5.9%

**Samples tested negative before study inclusion.*

PEER REVIEWED

Regardless of whether HPT is used in post-treatment testing, dogs should be tested for both antigen and microfilariae 6 to 12 months after completion of adulticidal therapy to assess treatment efficacy.

Many factors can affect detection of heartworm antigen in samples, leading to NAD results in truly infected dogs. They include the stage of infection, concurrent medications (including doxycycline and heartworm preventives), and microfilarial status of the animal (**Table 2**).

Stage of Infection

Although detection of *D immitis* is typically expected 7 months after infection (with earliest detection 5 months after infection),[7] some evidence suggests that HPT may allow earlier antigen detection.

- In one study of experimentally infected dogs, 100% of infections were detected after HPT of test samples obtained 4 months after infection.[8] When samples were obtained 5 months after infection, only 42.6% of infections were detected in samples without HPT, while 100% of those infections were identified after HPT.[8]
- In another study of experimental infection in dogs, positive antigen test results were obtained 31 to 36 days sooner in heated (days 127 to 132 after infection) versus unheated (day 163 after infection) serum samples.[9]
- In a study of experimental infection in cats, detection of heartworm antigen was possible as early as 5.5 months after infection when samples underwent HPT.[5]

The potential for earlier detection of heartworm antigen after HPT of samples from recently infected animals is theorized to be the result of a more robust immune response early after infection, along with the lower level of antigen produced by nongravid female worms, which both lead to greater immune complex formation and subsequent antigen blocking.

Heartworm Treatments

Several studies have suggested that the administration of macrocyclic lactones and/or antibiotic therapy (eg, doxycycline) can affect immune complex formation and subsequently interfere with antigen testing.

- A study of 19 naturally infected dogs being managed with monthly topical 10% imidacloprid plus 2.5% moxidectin along with oral doxycycline (10 mg/kg q12h for 30 days, every 6 months) demonstrated a substantial variation in timing of antigen detection between HPT and non-HPT samples.[10] Among dogs that initially tested negative with non-HPT samples, antigen was detected in 50%, 95%, and 100% of dogs tested with HPT after 6, 12, and 18 months of therapy, respectively.
- Another report of 29 shelter dogs with antigen detection after HPT demonstrated 3.8 times greater odds of immune complex interference with test results when a history of macrocyclic lactone administration was reported.[2]
- Fifty-three percent of a cohort of privately owned dogs that had a negative antigen test result and received monthly macrocyclic lactones and doxycycline had detectable antigen after HPT of serum samples.[3]

There are a few possible explanations for the influence of macrocyclic lactone and doxycycline administration on immune complex interference with diagnostic test results. Although doxycycline has some anti-inflammatory activity, administration of macrocyclic lactones and doxycycline in dogs with active heartworm infection is not adulticidal in the short term. The persistence of live heartworms allows for continued antigenic stimulation, subsequent inflammatory response, and antibody production in the face of a decreasing antigen load. Secondarily, use of these medications often results in sterilization of the female worms and subsequent decrease in antigen release. These factors could contribute to a relative antibody

TABLE 2 Effect of Heat Pretreatment on Clinical Factors

CLINICAL FACTOR	IMPACT OF HEAT PRETREATMENT	RATIONALE
Stage of infection	May allow earlier antigen detection	Immune response may be more robust immediately after infection, resulting in increased antibody production
Concurrent treatments (macrocyclic lactones, doxycycline)	May reduce frequency of negative test results in infected animals	Prolonged infection promotes continued inflammatory response in face of decreased antigen load, disrupting antigen-to-antibody ratio
Microfilariae	May reduce frequency of negative test results in infected animals	Circulating microfilariae promote continued inflammatory response and continued antibody formation

PEER REVIEWED

excess, disrupting the normal clearance mechanisms and promoting continued immune complex creation.

Presence of Microfilariae

The presence of detectable circulating microfilariae in canine blood samples also appears to influence the interference of immune complexes with antigen detection. Circulating microfilariae contribute to the chronic inflammatory response of heartworm disease and are therefore theorized to contribute to continued antibody production, allowing continued immune complex formation. One report of 26 shelter dogs with circulating microfilariae demonstrated 32 times greater odds of converting to a positive antigen test result after HPT compared with dogs that tested antigen negative both before and after HPT.[2] Other reports have also identified circulating microfilariae in dogs whose test results converted from negative to positive after HPT.[1,3,4] Interestingly, one of these reports included microfilariae of *D immitis*, *Dirofilaria repens*, and *Acanthocheilonema reconditum*,[1] which suggests the potential for decreased specificity of antigen tests after HPT.

CAN I PERFORM HEAT PRETREATMENT IN MY CLINIC?

A variety of veterinary diagnostic laboratories offer HPT panels upon request. In general, costs are minimal (<$30) and results are available within 1 to 3 days. Perhaps the biggest benefit of using diagnostic laboratories for HPT is the consistency and reliability of sample handling and testing techniques. Testing methods are typically validated and concomitantly run with positive controls to ensure accuracy of results.

Diagnostic laboratory services are preferred, but when these are not available or feasible, a simplified HPT protocol has been successfully used (**Box 1**, **Figure 2**). In a study of shelter dogs, 616 samples underwent the simplified HPT protocol.[2] Of these, 13 samples could not undergo repeat testing after heating. Five "untestable" samples were presumed to be directly related to inconsistencies in the application of heat, resulting in solidification of the serum sample. The remaining 8 samples were untestable for a variety of reasons presumably unrelated to the heating protocol (eg, insufficient serum, invalid controls).

WHEN SHOULD I CONSIDER HEAT PRETREATMENT?

Heat pretreatment is likely not indicated in most heartworm screening test scenarios. It should be considered when screening test results conflict (eg, antigen negative and microfilariae positive), when patients are receiving an alternative treatment protocol (eg, macrocyclic lactone and/or doxycycline), when the patient has known chronic inflammatory diseases (eg, pyoderma, otitis, endoparasitism), or when dogs test negative but originate from a known heartworm-endemic region and have a history of lapsed or no preventive administration. An NAD result on a pretreated sample by a diagnostic laboratory can also rule out suspicion of heartworm infection.

BOX 1.
Simplified Heat Pretreatment Protocol

1. Dilute serum sample with approximately equal volume of 0.9% NaCl.
2. Place ~250 mL tap water into 500-mL glass beaker.
3. Microwave beaker to the point of boiling (approximately 2 min in 1000 W).
4. Remove heated water from microwave and place tube with diluted sample in the heated water for 10 minutes. (Note: Place serum in a glass collection tube and remove the rubber stopper before heating. Some warping of tube is expected.)
5. Repeat antigen test.

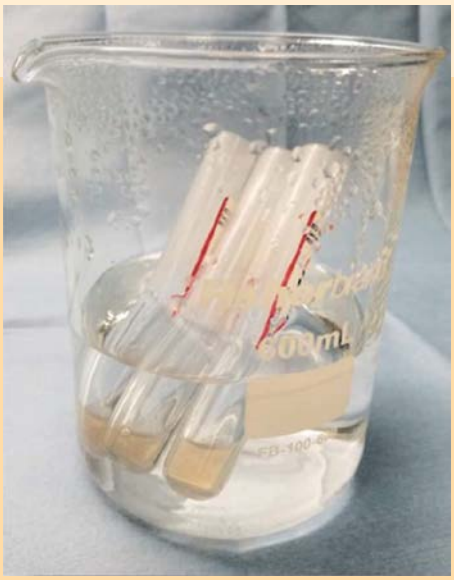


FIGURE 2. Diluted serum samples undergo a simplified heat pretreatment protocol. Note the use of glass tubes and the absence of rubber stoppers.

PEER REVIEWED

Brian DiGangi

Brian DiGangi, DVM, MS, DABVP, is a senior director of shelter medicine for the ASPCA. He earned his DVM from the University of Florida and is board certified in both canine and feline practice and shelter medicine practice. He is a member of the board of directors of the Association of Shelter Veterinarians and the American Heartworm Society. Dr. DiGangi has published research on feline adoption, canine heartworm disease, and immunology. Prior to joining the ASPCA, he served as a clinical associate professor at the University of Florida.

In short, clinicians should consider HPT—as well as clinical staging with physical examination, complete blood count, blood chemistry analysis, urinalysis, and radiography—whenever there is a strong clinical suspicion of heartworm disease in the presence of negative screening test results. One report identified heartworm antigen in 64.7% of "negative" samples from patients for which the veterinarian's clinical suspicion strongly supported heartworm infection.

The bottom line: There is no substitute for the clinical acumen of a veterinarian.

SUMMARY

Immune complex interference is one factor clinicians should consider when interpreting the results of diagnostic tests that rely on antigen detection, especially when screening test results do not match clinical suspicions. Recent research has provided some insight into factors that can affect screening test results while identifying specific scenarios that may justify the added step of HPT of serum samples. These findings highlight the importance of adhering to the American Heartworm Society's diagnostic testing recommendations. Annual screening for both antigen and microfilariae is the best way to identify heartworm infection as early and as consistently as possible. **TVP**

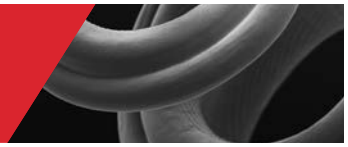
To see the references for this article, please visit **tvpjournal.com**.

ERRATUM

"Heartworm Disease: The Science, The Practice, The Future: A Selective Summary of the 15th Triennial Heartworm Symposium" by Dr. Clarke Atkins, published in the March 2017 issue of the AHS Bulletin (Vol. 44, No. 1). The article was originally published in *Today's Veterinary Practice* 2017;7(1):87-92 and was reprinted with permission.

The author regrets that an error appeared in the dosage of ivermectin used in a microfilaria suppression test on pages 8 and 9. The dosage should be 50 µg/kg, not 50 mg/kg. The corrected section is provided below:

A new approach to identification or resistance in suspect cases was presented. Practitioners concerned that they may be seeing resistant cases in their practices can use a new clinical decision tree created at the University of Georgia College of Veterinary Medicine. The step-by-step process includes careful review of the case records to verify gaps in prevention. If suspicion persists, practitioners are advised to quantitate microfilariae and then perform a microfilaria suppression test. The test administers ivermectin (50 µg/kg) or milbemycin (1 mg/kg) with retesting 7 days later. If microfilariae are eliminated, the odds of a resistant biotype being involved are extremely low. Meanwhile, when microfilariae are not significantly suppressed (< 75% reduction), the suspicion of resistance increases.



MINIMIZING HEARTWORM TRANSMISSION IN RELOCATED DOGS

Editor's note: The following document was developed by the American Heartworm Society in collaboration with the Association of Shelter Veterinarians.

Transporting and relocating dogs is an increasingly common practice. Whether the situation is an owned pet accompanying emigrating or traveling caretakers, the relocation of homeless animals for adoption, or the movement of dogs for competition, exhibition, research or sale, this process carries the risk of spreading infectious diseases. This includes the transmission of *Dirofilaria immitis* when infected dogs have become microfilaremic.

The following practices will minimize the risk of heartworm transmission associated with the transportation and relocation of dogs (see also Algorithm):

1. **Test all dogs greater than 6 months of age** for microfilariae (Mf) and heartworm antigen (Ag) prior to relocation.
   a. If testing is not possible, assume transmission is possible and proceed to Step 3b.

2. **If dogs test positive** for microfilariae or antigen, reconsider relocation at this time and begin treatment in accordance with the American Heartworm Society (AHS) Guidelines.
   a. Dogs with clinical signs attributed to heartworm infection should not be transported.
   b. Dogs that have been treated with melarsomine dihydrochloride should not be transported for at least 4 weeks after an injection to minimize stress and physical exertion that accompany the relocation process.

3. **If dogs test positive** and relocation cannot be postponed, clinical decisions should be based on the dog's heartworm status.
   a. If Mf-, Ag+:
      i. Administer an approved macrocyclic lactone product.
      This should prevent the pre-patent dog from becoming microfilaria positive.[1]
      ii. Begin doxycycline therapy.
      A 4-week course of doxycycline should prevent the pre-patent dog from becoming microfilaria positive.[2,3]
      iii. Repeat Knott's testing in 7 days; if negative, proceed with relocation. If positive, repeat Knott's testing in 7 days.
      Two negative tests 7 days apart can provide reasonable assurance of a lack of circulating microfilariae and reduced risk of transmission.
   b. If Mf+, Ag- OR Mf+, Ag+:
      i. Apply an approved moxidectin topical product, proceed with relocation.
      A single dose of topical moxidectin prior to transport will eliminate most microfilariae.[4-6]
      OR
      i. Administer an approved macrocyclic lactone product along with a topical canine insecticide (containing permethrin + dinotefuran + pyriproxyfen) that is labeled to kill and repel mosquitoes.
      This will prevent infection of mosquitoes and subsequent transmission of infective larvae during transportation and for 1 month thereafter.[7]
      ii. Begin doxycycline therapy.
      Administration of a 4-week course of doxycycline will render microfilariae incapable of normal development to infective larvae in mosquitoes and subsequent development of these larvae in dogs.[8,9]

Once heartworm-positive dogs have been safely transported, heartworm treatment should be completed according to AHS Guidelines as soon as possible.

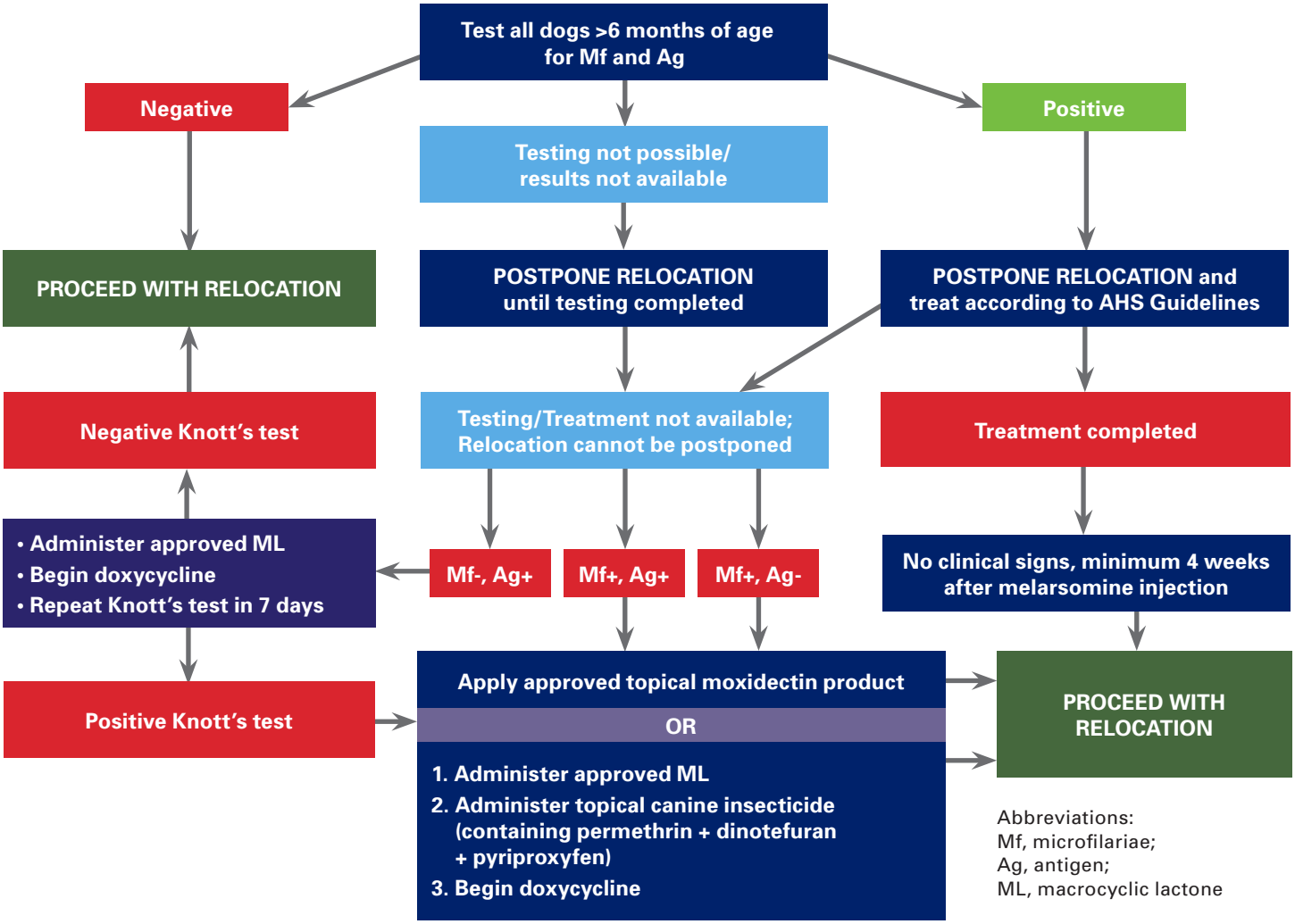
4. **If dogs test negative** for microfilariae and antigen, proceed with relocation.
   a. Administer macrocyclic lactone preventive drugs to dogs greater than 6 weeks of age prior to relocation.[1]
   b. Repeat microfilariae and antigen testing in 6 months. If a history of preventive administration is well documented, repeat testing in 12 months.[1]

Caring for dogs that undergo relocation is an everyday challenge veterinarians face in today's mobile society, and one that necessitates the adoption of approaches to mitigate heartworm transmission. Along with considering the recommendations in this document, veterinarians should ensure that transportation of animals is carried out in accordance with state and/or federal transportation regulations, as well as professional guidelines.[10,11] In the case of organized homeless animal relocation programs, veterinarians should work with both source and destination organizations to establish protocols for minimizing transmission of infectious diseases, including heartworm disease.

Continues next page.

Minimizing Heartworm Transmission continued from page 19

ALGORITHM FOR MINIMIZING HEARTWORM TRANSMISSION IN RELOCATED DOGS



Abbreviations:
Mf, microfilariae;
Ag, antigen;
ML, macrocyclic lactone

References

1. American Heartworm Society. Current Canine Guidelines for the Prevention, Management, and Diagnosis of Heartworm Infection in Dogs. Available online at: https://heartwormsociety.org/images/pdf/2014-AHS-Canine-Guidelines.pdf
2. Hoerauf A, Mand S, Fischer K, et al. Doxycycline as a novel strategy against bancroftian filariasis – depletion of *Wolbachia* endosymbionts from Wuchereria bancrofti and stop of microfilaria production. *Med Microbiol Immunol*. 2003; 192:211-216.
3. Rossi MID, Paiva J, Bendas A, et al. Effects of doxycycline on the endosymbiont *Wolbachia* in *Dirofilaria immitis* (Leidy, 1856) — Naturally infected dogs. *Vet Parasitol*. 2010; 174:119-123.
4. Bayer Healthcare LLC. Advantage Multi® Product Label. Available online at: https://bayer.naccvp.com/product/view/basic/1040052?u=bayer&p=dvm
5. Blagburn BL, Dillon AR, Arther RG, et al. Comparative efficacy of four commercially available heartworm preventive products against the MP3 laboratory strain of *Dirofilaria immitis*. *Vet Parasitol*. 2011; 176:189-194.
6. McCall JW, Arther R, Davis W, Settje T. Safety and efficacy of 10% imidacloprid + 2.5% moxidectin for the treatment of *Dirofilaria immitis* circulating microfilariae in experimentally infected dogs. *Vet Parasitol*. 2014; 206; 5-13.
7. McCall JW, Hodgkins E, Varloud M, et al. Blocking the transmission of heartworm (*Dirofilaria immitis*) to mosquitoes (*Aedes aegypti*) by weekly exposure for one month to microfilaremic dogs treated once topically with dinotefuran-permethrin-pyriproxyfen. In Proceedings, 2016 American Heartworm Society Triennial Symposium. *Parasites & Vectors*, 2017, in press.
8. McCall JW, Genchi C, Kramer L, et al. Heartworm and *Wolbachia*: therapeutic implications. *Vet Parasitol*. 2008; 158:204-244.
9. McCall JW, Kramer L, Genchi C, et al. Effects of doxycycline on heartworm embryogenesis, transmission, circulating microfilaria, and adult worms in microfilaremic dogs. *Vet Parasitol*. 2014; 206(1-2):5-13.
10. American Veterinary Medical Association. Relocation of Dogs and Cats for Adoption: Best Practices. Available online at: https://www.avma.org/KB/Resources/Reference/AnimalWelfare/Documents/AVMA_BestPracticesAdoption_Brochure.pdf
11. Association of Shelter Veterinarians. Guidelines for Standards of Care in Animal Shelters. Available online at: http://www.shelter-vet.org/assets/docs/shelter-standards-oct2011-wforward.pdf

WELCOME TO OUR 2017–2018 AHS STUDENT LIAISONS

As part of our ongoing efforts to fulfill the mission of the American Heartworm Society—to lead the veterinary profession and the public in the understanding of heartworm disease—AHS has established a student liaison program to increase awareness of who we are and the many resources we offer. By engaging these future practitioners, we hope to develop a new generation of leaders in the fight against heartworm.

The student liaisons will be representing AHS at vet school events, including local CE events, open houses, and community fairs. They will also distribute AHS promotional information and will present at meetings of the Student Chapters of the American Veterinary Medical Association (SCAVMA). Each student will serve a one-year term, but may apply to continue.

As an incentive to serve, student liaisons will receive free registration to the AHS Triennial Symposium and an AHS fleece vest and nametag. Participation also provides an opportunity to network and is a great resume builder.

Please welcome our first group of student liaisons:

Auburn University	Allison Siu
UC Davis	Kelsey Broadhead
University of Georgia	Jordyn Whitfield
Lincoln Memorial University	Whitney Patz
Louisiana State University	Rachel Pool
Louisiana State University	Alexandra Ford
Michigan State University	Bethany Myers
Mississippi State University	Anna Walker
North Carolina State University	Kelly Hood
North Carolina State University	Karolina Szewczyk
North Carolina State University	Amie Pflaum
North Carolina State University	Caroline Brewer
North Carolina State University	Shanna Wong
The Ohio State University	Natalie Vasquez
The Ohio State University	Olivia Stephenson
Oklahoma State University	Samantha Hancock
Oklahoma State University	Josiah Dame
Oregon State University	Stacie Nellor
University of Pennsylvania	Steve Hanes
Purdue University	To be named
Ross University	McKinsey Landers
Royal Veterinary College	Amanda Sircy
St. George's University	Jocelyn Renfro
St. George's University	Monica Tetnowski
St. Matthews University	Selina Morales Cornell
Tufts Cummings School of Vet Med	David Krucik
Utah (WIMU)*/ USU-WSU	Ariel Nelson
Utah (WIMU)*/ USU-WSU	Sarah Frandsen
University of Wisconsin–Madison	Jenna Motz
University of Wisconsin–Madison	Lindsay Dillon

*WIMU = Washington, Idaho, Montana, Utah

Brief Summary of Prescribing Information

DIROBAN™

Canine Heartworm Treatment

Sterile Powder for Injection

CAUTION : Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

WARNING
DIROBAN should be administered by deep intramuscular injection ONLY in the epaxial (lumbar) muscles (L_3 - L_5).
DO NOT USE IN ANY OTHER MUSCLE GROUP. DO NOT USE INTRAVENOUSLY.
Care should be taken to avoid superficial injection or leakage (see SAFETY).

INDICATIONS

DIROBAN Sterile Powder for Injection is indicated for the treatment of stabilized Class 1[a], 2[b], and 3[c] heartworm disease caused by immature (4 month-old, stage L_5) to mature adult infections of *Dirofilaria immitis* in dogs.

Heartworm Disease Classification: The following parameters were used to classify the dogs in the clinical field trials for DIROBAN. Other parameters may be considered. As a general rule, conservative treatment should be employed since heartworm disease is serious and potentially fatal. If there is evidence of a high worm burden, patients should be categorized as Class 3.

[a] Class 1: Patients in this category are characterized as having asymptomatic to mild heartworm disease. No radiographic signs or signs of anemia are evident. Patients with mild disease may have subjective signs such as a general loss of condition, fatigue on exercise, or occasional cough; however, no objective radiographic or other abnormal laboratory parameters will be present.

[b] Class 2: Patients in this category are characterized as having moderate heartworm disease. Radiographic signs or signs of anemia [Packed Cell Volume (PCV) less than 30% but greater than 20%, or other hematologic parameters below normal] are evident. Mild proteinuria (2+) may be present. Radiographic signs may include right ventricular enlargement, slight pulmonary artery enlargement, or circumscribed perivascular densities plus mixed alveolar/interstitial lesions. Patients may be free of subjective clinical signs or may have a general loss of condition, fatigue on exercise, or occasional cough. If necessary, patients should be stabilized prior to treatment.

[c] Class 3: Patients in this category are characterized as having severe heartworm disease. These patients have a guarded prognosis. Subjective signs of disease may include cardiac cachexia (wasting), constant fatigue, persistent cough, dyspnea, or other signs associated with right heart failure such as ascites and/or jugular pulse. Radiographic signs may include right ventricular enlargement or right ventricular plus right atrial enlargement, severe pulmonary artery enlargement, circumscribed to chronic mixed patterns and diffuse patterns of pulmonary densities or radiographic signs of thromboembolism. Signs of significant anemia (PCV <20% or other hematologic abnormalities) may be present. Proteinuria (> 2+) may be present. Patients may have only moderate clinical signs and significant laboratory or radiographic alterations or they may have significant clinical signs with only moderate laboratory and radiographic signs and be categorized as Class 3. Patients in Class 3 should be stabilized prior to treatment and then administered the alternate dosing regime (see **PRECAUTIONS and DOSAGE AND ADMINISTRATION**).

CONTRAINDICATIONS

DIROBAN is contraindicated in dogs with very severe (Class 4) heartworm disease. Patients in this category have Caval Syndrome (*D. immitis* present in the venae cavae and right atrium).

WARNINGS

(See boxed Warning.) For use in dogs only. Safety for use in breeding animals and lactating or pregnant bitches has not been determined.

HUMAN WARNINGS

Keep this and all medications out of the reach of children. Avoid human exposure. Wash hands thoroughly after use or wear gloves. Potentially irritating to eyes. Rinse eyes with copious amounts of water if exposed. Consult a physician in cases of accidental exposure by any route (dermal, oral, or by injection).

The Safety Data Sheet (SDS) contains more detailed occupational safety information. To report adverse effects, obtain a SDS or for assistance, contact Zoetis Inc. at 1-888-963-8471.

PRECAUTIONS

General: All dogs with heartworm disease are at risk for post-treatment pulmonary thromboembolism (death of worms which may result in fever, weakness, and coughing), though dogs with severe pulmonary arterial disease have an increased risk and may exhibit more severe signs (dyspnea, hemoptysis, right heart failure and possibly death). Dogs should be restricted from light to heavy exercise post-treatment depending on the severity of their heartworm disease.

Studies in healthy (heartworm negative) dogs indicate that adverse reactions may occur after the second injection in the series even if no problems were encountered with the first injection. All patients should be closely monitored during treatment and for up to 24 hours after the last injection.

Special Considerations for Class 3 dogs: Following stabilization, severely ill (Class 3) dogs should be treated according to the alternate dosing regime in an attempt to decrease post-treatment mortality associated with thromboembolism (see **DOSAGE AND ADMINISTRATION**). Post-treatment mortality due to thromboembolism and/or progression of the underlying disease may occur in 10 to 20% of the Class 3 patients treated with DIROBAN (see ***Mortality***). Hospitalization post-treatment and strict exercise restriction are recommended. Other supportive therapies should be considered on a case-by-case basis.

If the alternate dosing regime is used, expect increased injection site reactions on the side receiving the second injection since the skeletal muscles at the first injection site may not have fully recovered (healed). If persistent swelling is present at 1 month, the second injections may be delayed for several weeks up to 1 month.

Special Considerations for Older Dogs: In clinical field trials, dogs 8 years or older experienced more post-treatment depression/lethargy, anorexia/inappetence, and vomiting than younger dogs.

ADVERSE REACTIONS (SIDE EFFECTS)

Injection Sites: At the recommended dosage in clinical field trials, significant irritation was observed at the intramuscular injection sites, accompanied by pain, swelling, tenderness, and reluctance to move. Approximately 30% of treated dogs experienced some kind of reaction at the injection site(s). Though injection site reactions were generally mild to moderate in severity and recovery occurred in 1 week to 1 month, severe reactions did occur (< 1.0%), so care should be taken to avoid superficial or subcutaneous injection and leakage. Firm nodules can persist indefinitely.

Other Reactions: Coughing/gagging, depression/lethargy, anorexia/inappetence, fever, lung congestion, and vomiting were the most common reactions observed in dogs treated with melarsomine dihydrochloride. Hypersalivation and panting occurred rarely in clinical trials (1.9% and 1.6%, respectively); however, these signs may occur within 30 minutes of injection and may be severe. One dog vomited after each injection of melarsomine dihydrochloride, despite pretreatment with anti-emetics. All adverse reactions resolved with time or treatment with the exception of a limited number of injection site reactions (persistent nodules, (see Table: **Average Onset Time and Duration (with Ranges) of the Most Common Reactions in Clinical Trials)** and a low number of post-treatment deaths (see ***Mortality***).

Prevalence of Clinical Observations/Adverse Reactions Reported in Clinical Field Trials: The following table enumerates adverse events that occurred in 1.5% or more of dogs with Class 1, 2, and 3 heartworm disease treated with melarsomine dihydrochloride in clinical field trials. Comparison is made with the same adverse events reported in dogs treated with placebo. Some of the following clinical observations/adverse reactions seen in dogs treated with melarsomine dihydrochloride may be directly attributable to the drug or they may be secondary to worm death and/or the underlying heartworm disease process.

Prevalence of Clinical Observation/Adverse Reactions Reported in Clinical Field Trials

Clinical Observation/ Adverse Reaction	Melarsomine dihydrochloride % of dogs n=311	PLACEBO % of dogs n=63
Injection Site Reactions	32.8	3.2
Coughing/Gagging	22.2	14.3
Depression/Lethargy	15.4	4.8
Anorexia/Inappetence	13.2	3.2
Pyrexia (fever)	7.4	0.0
Lung Congestion/Sounds	5.5	1.6
Emesis	5.1	1.6
Diarrhea	2.6	0.0
Dyspnea	2.6	1.6
Hypersalivation	1.9	0.0
Panting	1.6	0.0
Hemoptysis	1.6	0.0

Clinical observations/adverse reactions occurring in less than 1.5% of the dogs treated with melarsomine dihydrochloride include: abdominal hemorrhage, abdominal pain, bloody stool/diarrhea, colitis, gingivitis, pancreatitis, anemia, DIC, hemoglobinemia, icterus (mucous membranes), discolored urine, hematuria, inappropriate urination, low specific gravity, polyuria, pyuria, bronchitis, miscellaneous respiratory problem, pneumonia, tachypnea, tracheobronchitis, wheezing, alopecia, hair color and coat character change, miscellaneous skin problem, ataxia, disorientation, fatigue/tires easily, miscellaneous eye problem, weight loss, convulsion/seizure, leukocytosis, polydipsia, and restlessness.

Onset and Duration of Clinical Observations/Adverse Reactions: The following table is provided to show the average onset time post-treatment for the most common reactions and the average duration of each event, as calculated from the 311 dogs treated with melarsomine dihydrochloride in the clinical field trials.

Average Onset Time and Duration (with Ranges) of the Most Common Reactions in Clinical Trials

Clinical Observation/Adverse Reaction	Avg. Onset Time in Days (range)*	Avg. Duration in Days (range)*
Injection Site		
Swelling/Edema/Seroma	6 (0*-77)	18 (< 1-210)
Pain/Discomfort/ Irritation/Inflammation/Heat	1 (0-6)	3 (< 1-30)
Generalized/Local Myalgia with Tenderness and Stiffness	3 (1-8)	9 (< 1-30)
Persistent (lumps, knots, nodules, masses)	22 (0-99)	47 (1-152)
Abscess (sterile and septic)	24 (10-42)	21 (5-36)
Coughing/Gagging	10 (0-103)	13 (< 1-134)
Depression/Lethargy	5 (0-46)	6 (< 1-48)
Anorexia/Inappetence	5 (0-63)	5 (< 1-30)

*A zero indicates that the reaction first occurred on the day of treatment.

Mortality: Death is a possible sequelae of heartworm disease in dogs with or without treatment, especially in the Class 3 dogs. The following table shows the percentage of dogs that died in clinical trials with melarsomine dihydrochloride and the causes of death, if known.

Mortality in Dogs with Class 1, 2, and 3 Heartworm Disease Treated with melarsomine dihydrochloride in Clinical Field Trials

	CLASS 1, 2 % OF DOGS n=267	CLASS 3 % OF DOGS n=44
Total Deaths	5.2	18.2
Cause:		
Trauma	2.3	2.3
Thromboembolism	0.0	4.6
Euthanasia (unrelated to treatment or underlying disease)	1.1	0.0
Euthanasia (related to treatment or underlying disease)	0.0	2.3
Underlying Disease	0.8	2.3
Undetermined	1.1	6.8

In one small (n=15), uncontrolled field study in severely ill (Class 3) dogs, 5 dogs died following treatment. Pulmonary thromboembolism was the cause of one death. The remaining dogs were not necropsied. All 5 dogs were in right heart failure at the time of treatment. Clinical signs seen in this study which were not seen in the larger studies include atrial fibrillation, collapse, hypothermia, and weakness.

Post Approval Experience: In addition to the aforementioned adverse reactions reported in pre-approval clinical studies, there have also been rare reports of paresis and paralysis in dogs following administration of melarsomine dihydrochloride. To report a suspected adverse reaction, contact Zoetis Inc. at 1-888-963-8471.

Overdosage: Three dogs were inadvertently overdosed with melarsomine dihydrochloride in the clinical field trials when the dose was calculated on a mg/lb basis rather than a mg/kg basis (2X overdosage). Within 30 minutes of injection, one dog showed excessive salivation, panting, restlessness, and fever with all signs resolving within 4 hours. Vomiting and diarrhea were seen in the second dog within 24 hours of injection. The dog vomited once and the diarrhea resolved within 24 hours. The third dog showed no systemic reaction to the overdosage. Clinical observations in healthy beagle dogs after receiving up to 3X the recommended dose included tremors, lethargy, unsteadiness/ataxia, restlessness, panting, shallow and labored respiration, rales, severe salivation, and vomiting which progressed to respiratory distress, collapse, cyanosis, stupor, and death (see **SAFETY**).

BAL in Oil Ampules (Dimercaprol Injection, USP) [Akorn, San Clemente, California, at 1-800-223-9851] is reported in the literature to be an antidote for arsenic toxicity and was shown in one study to reduce the signs of toxicity associated with overdosage of melarsomine dihydrochloride. The efficacy of melarsomine dihydrochloride may be reduced with co-administration of BAL.

STORAGE CONDITIONS

Store upright at room temperature (15° - 30°C). After reconstitution, solutions should be stored under refrigeration and kept from light in the original packaging for 36 hours. Do not freeze reconstituted solution.

HOW SUPPLIED

DIROBAN is provided as 5 - 50 mg vials of lyophilized melarsomine dihydrochloride with accompanying 5 - 2 mL vials of sterile water for injection USP.

ANADA 200-609, Approved by FDA

zoetis

Distributed by:
Zoetis Inc.
Kalamazoo, MI 49007

August 2016

30559700A&P

[1] Data on file at Merial.
[2] Freedom of Information: NADA140-971 (January 15, 1993).

MERIAL

IMPORTANT SAFETY INFORMATION: HEARTGARD® Plus (ivermectin/pyrantel) is well tolerated. All dogs should be tested for heartworm infection before starting a preventive program. Following the use of HEARTGARD Plus, digestive and neurological side effects have rarely been reported. For more information, please visit www.HEARTGARD.com.

Heartgard® Plus
(ivermectin/pyrantel)

UC DAVIS VETERINARIANS REMOVE 13-cm HEARTWORM FROM CAT'S FEMORAL ARTERY

Removal of a heartworm via the femoral artery is extremely rare in veterinary medicine. Reported on only a few occasions in dogs, this is the first known report in cats.

From a press release from UC Davis on July 3, 2017–

Stormie, a 4-year-old female Siamese cat, has had a history of heartworm disease since she was adopted at 1 year of age. She and her owner live in Los Angeles, but were visiting family in the Bay Area when she developed pelvic limb lameness. Fearing she had fallen off something or down a staircase, Stormie's owner brought her to a local veterinary emergency room in Berkeley, where she informed them of the history of heartworm disease. After ultrasound showed a suspected heartworm in the arterial system, and a heartworm antigen test resulted in a strong positive, Stormie's owner was advised to bring her to specialists at the UC Davis veterinary hospital.

Once at UC Davis, the Cardiology Service responded to Stormie's emergency arrival, and confirmed the referring veterinarian's diagnosis. To get a better idea of the exact location of the worm and to form a treatment plan, the cardiologists performed an echocardiogram. The imaging test revealed a heartworm in the pulmonary artery. They also saw evidence of pulmonary hypertension from the heartworm disease.

An abdominal ultrasound followed, and confirmed that the heartworm extended into her abdominal aorta and down her leg into the right femoral

Continues on page 27.

WHAT'S NEW FROM AHS THIS FALL?

A QUARTERLY UPDATE ON AHS PROGRAMS AND RESOURCES

AMERICAN HEARTWORM SOCIETY™
EST. 1974

AHS IN THE NEWS

In 2017, we are continuing our "Heartworm Hotline" series in *Today's Veterinary Practice*. The July/August article, which was authored by AHS board member Dr. Brian DiGangi, covered the role of heat pretreatment of serum samples in heartworm diagnosis, and is reprinted in this issue. The September/October issue will feature an expanded summary of the AHS incidence survey results, and will be co-authored by AHS President Dr. Chris Rehm and long-time board member and survey administrator Dr. Doug Carithers. The November/December article, which will be authored by board member Dr. Andy Moorhead, will focus on Wolbachia and heartworm, as well as why doxycycline is needed in heartworm treatment.

The "AHS Quarterly" series continues in *Clinician's Brief*. The June issue, by Dr. Chris Rehm, outlined the findings of the new AHS incidence map and survey.

Think12
AMERICAN HEARTWORM SOCIETY™

We have a new series of four new posters that can be downloaded and printed OR used in social media posts. The focus of these posters is the message that the risk of heartworm is real. These have already performed well on social media posts—in particular, the poster that depicts real heartworms; this image alone has been "liked," commented on and shared by thousands of members of our Facebook community. These new posters can be downloaded at https://heartwormsociety.org/veterinary-resources/practice-tools/posters

We also recently introduced a new slide show that lists 12 ways to protect pets from heartworm 12 months a year. Be sure to check out and share these new tools.

NEW HEARTWORM BROCHURE IN AHS-ASV SERIES FOR PET ADOPTERS

The Heartworm Disease Resource Task Force, a partnership between The Association of Shelter Veterinarians (ASV) and American Heartworm Society (AHS), has released the sixth and final brochure—on feline heartworm disease—in a series for people adopting a pet from a shelter. These illustrated, professionally designed brochures are available for download from https://heartwormsociety.org/veterinary-resources/shelter-resources.

AHS GUIDELINES NOW AVAILABLE IN CHINESE

Translations of the most recent canine and feline guidelines are now available in both Traditional and Simplified Chinese. These guidelines, as well as other translations in Spanish, French, Italian, and Portuguese (canine only), can be downloaded at https://heartwormsociety.org/veterinary-resources/american-heartworm-society-guidelines.

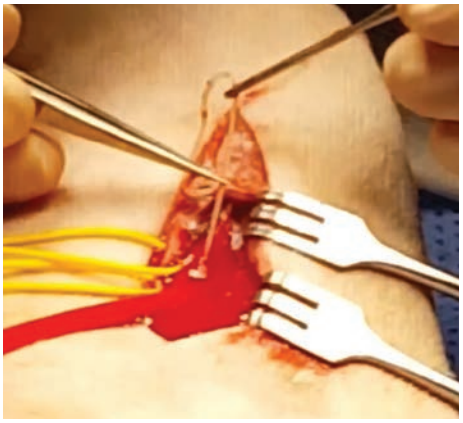
2016 TRIENNIAL SYMPOSIUM PROCEEDINGS

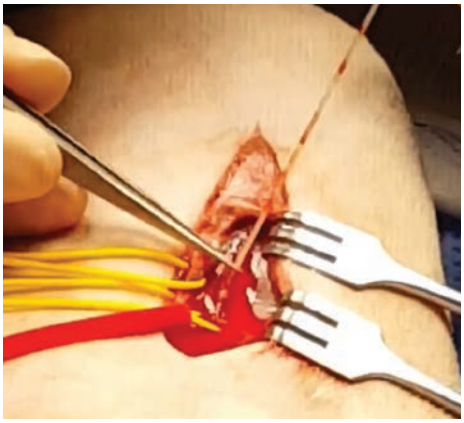
Twenty-nine manuscripts were submitted to *Parasites & Vectors* in July after a lengthy peer review, revision, and copy-editing process. This is 12 more papers than published in past proceedings! Five additional manuscripts went through the review process. We are awaiting word from *Parasites & Vectors* on publication date.

AHS BOARD MEETS IN OCTOBER

The next board meeting will be held October 29–31. A key area of discussion will be the AHS Guidelines and whether updates are needed, based on data and information that will be newly published in the 2016 AHS Triennial Symposium proceedings.

Feline Heartworm Removal continued from page 25.





artery. The worm was cutting off blood supply to the right leg and needed to be addressed immediately in order to avoid amputation.

Members of the Anesthesia/Critical Patient Care and Diagnostic Imaging services placed Stormie under general anesthesia and performed a CT angiography scan. The scan did not reveal any additional heartworms, but revealed there were abnormalities in the soft tissues in the right back leg likely secondary to decreased blood flow from the worm. It also showed evidence of inflammation in the lungs, which was likely also caused by the heartworms.

Cardiologist Dr. Catherine Gunther-Harrington and Dr. Ingrid Balsa of the Soft Tissue Surgery Service, assisted by cardiology resident Dr. Maureen Oldach, collaborated to successfully remove the 13-centimeter heartworm from Stormie's right femoral artery without breaking it. Because there was normal blood flow through the artery once the worm was removed—and the leg tissue still looked healthy—the artery was repaired, and the doctors decided that amputation was not necessary. However, Stormie's leg may require amputation in the future if the nerves and muscle in that leg do not heal properly, which may take months.

Stormie stayed hospitalized for four days so the Intensive Care Unit team could closely monitor her recovery. In addition to painkillers and anti-inflammatory medications, she was given an antibiotic that will help weaken the remaining heartworms in her system. As the worms die, they will break up into small pieces that could lodge in her lungs or other places throughout the circulatory system. In order to prevent this, she was prescribed a medication that will help break up blood clots and prevent new clots from forming. She was also placed on a monthly heartworm preventative that she will need to continue for the rest of her life. To avoid a future amputation, Stormie's owner has her in physical rehabilitation and is hopeful that she will continue to improve.

Removal of a heartworm via the femoral artery is extremely rare in veterinary medicine. It has been reported on only a few occasions in dogs, but never in cats. Due to this uniqueness, Dr. Oldach is currently preparing a case write-up for submission to a scientific journal.

A video of the procedure can be seen here: http://www.vetmed.ucdavis.edu/whatsnew/article.cfm?id=3884

AMERICAN HEARTWORM SOCIETY™
EST.1974

PO Box 8266, Wilmington, DE 19803-8266

First Class
U.S. Postage
PAID
Permit #270
Aurora, IL

Return Service Requested.