Effects of Doxycycline on Early Infections of *Dirofilaria immitis* and *Brugia pahangi* in Dogs with Dual Infections

J. W. McCall,[1] L. Kramer,[2] C. Genchi,[3] J. Guerrero,[4]
M. T. Dzimianski,[1] P. Supakorndej,[1] A. E. Mansour,[5]
S. D. McCall,[5] N. Supakorndej,[5] G. Grandi,[2] B. Carson[5]

2:10-2:25 pm • Saturday, April 17, 2010

Compelling evidence continues to mount in support of the hypothesis that there is a reciprocally dependent relationship between the *Wolbachia* endosymbionts and the filarial parasites that harbor them. *Wolbachia*-associated molecules have been implicated in the inflammatory response and immune tolerance, and they are thought to invoke an acquired immune response to these filariae.

In filarial parasites that harbor *Wolbachia*, every individual harbors some of the bacteria from birth, even though their numbers vary among individuals in each stage and population size varies widely among life cycle stages. Tetracycline treatment of animals and humans infected with filariae that harbor *Wolbachia* is considered to exert its antifilarial effects by killing the *Wolbachia*. *Wolbachia* population dynamics are consistent with (1) the two processes compromised most by tetracyclines (ie, molts from L3-L4 and L4-juvenile; microfilaria production) and (2) evasion of mammalian immunity, allowing long-term survival of adult filariae. Furthermore, survival of microfilaria after doxycycline treatment of heartworm-microfilaremic dogs, followed by normal development of these microfilaria to L3 and the inability of the L3 to develop to adults in recipient dogs (ie, blocks transmission) strongly suggest that relatively few *Wolbachia* are required for microfilaria to survive and develop to L3, but substantial numbers are needed for development of L3 to adults.

Earlier work in rodent filarial models has shown that L3, L4, and juvenile filariae are highly susceptible to tetracyclines. The primary objective of this study is to determine the effects of doxycycline on the larval and juvenile stages of *Dirofilaria immitis*, with a secondary objective of determining the effects of the drug on all stages of *Brugia pahangi* in dogs with dual infections of these two filariae. Twenty young male and female beagle dogs were allocated to 4 groups of 5 dogs each. Each dog was given 50 *D. immitis* L3 (inguinal area) and 200 *B. pahangi* (100 L3 in the dorsum of each hind paw) by subcutaneous injection. The dogs in groups 1-3 were given doxycycline orally at 10 mg/kg/day twice daily for a month at selected times in the life cycle of *D. immitis*. Group 1 received the drug on days 0-29 (L3 and early L4 for *D. immitis*; L3, L4 and L4-juvenile molt for *B. pahangi*). Group 2 received the drug on days 40-69 (L4-juvenile molt for *D. immitis*; microfilaria development for *B. pahangi*). Group 3 received the drug on days 65-94 (migration of *D. immitis* to the pulmonary arteries; patency for *B. pahangi*). Group 4 served as untreated controls. The dogs are being bled for microfilaria and antigen (Ag) testing at monthly intervals beginning at day 84 postinfection. At this time (day 166 – too early for *D. immitis* microfilariae), all control dogs have had high *B. pahangi* microfilaria counts for 3-4 months, while all dogs in group 3 have had a low-level, transient microfilaremia, and no microfilariae have been detected in groups 1 and 2. All control dogs and 1 dog in each of groups 1 and 3 are positive for *D. immitis* Ag, while none of the dogs in group 2 have been Ag-positive. Necropsy for recovery of adult worms (heart/pulmonary arteries, *D. immitis*; lymphatics, *B. pahangi*) to determine efficacy will be done at ~210 days postinfection. Some worms and selected tissue samples will be processed for molecular biology, immunohistochemistry (WSP), and histologic examination.

1 College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA
2 Universita` di Parma, 43100 Parma, Italy
3 Universita` degli Studi di Milano, Milano, Italy
4 School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA
5 TRS Labs, Inc., Athens, Georgia, USA

SATURDAY, APRIL 17 ■ SESSION 8

Diagnosis and Treatment Strategies: Impacting Prognosis, Efficacy, and Safety, Part II

Sponsored by MERIAL

Dirofilaria immitis Infection in Dogs: Biochemical Cardiopulmonary Marker Levels

E. Carretón,[1] J. A. Corbera,[1] M. C. Juste,[1] R. Morchón,[2]
F. Simón,[2] J. A. Montoya-Alonso[1]

3:20-3:35 pm • Saturday, April 17, 2010

Cardiopulmonary biomarkers are biological parameters that can be objectively measured and quantified as indicators of pathogenic processes (heartworm disease) or as responses to therapeutic intervention. Biomarkers are typically thought of as tools for the screening, diagnosis, or monitoring of the process of a disease, or for determining the prognosis. However, they may also be used to determine the eligibility of a disease for specific therapies and susceptibility of the aforementioned to those therapies.

The aim of the present study was to determine the levels of cardiopulmonary biomarkers (cardiac troponin T, myoglobin, and D-dimer) in dogs parasitized by *Dirofilaria immitis*. Seventy-one dogs were analyzed: 9 healthy animals (group 1), 16 seropositive and amicrofilaremic dogs (group 2), and 46 microfilaremic and seropositive dogs (group 3). All patients were companion animals that lived in a *Dirofilaria immitis*-endemic area, and none of them came from a population of animals used for research. The

measurement of circulating cardiopulmonary biomarkers concentration was performed in heparinized blood, using a specific troponin T, myoglobin, and D-dimer immunoassay system (Cardiac Reader®, Roche Diagnostics) following manufacturer instructions. In groups of dogs 1, 2, and 3, troponin T levels were below the detection limit of the equipment (<0.03 ng/mL). Group 1 showed myoglobin levels <30 ng/mL, and group 2 had a 6.25% higher level of myoglobin (>100 ng/mL). In group 3, 21.74% of dogs had pathological levels of myoglobin (>100 ng/mL). Group 1 presented levels of D-dimer <0.1 µg/mL. Fifty percent of the amicrofilaremic dogs (group 2) showed detectable levels of D-dimer between 0.1 and 0.5 µg/mL, which was not considered pathological; and 6.25% of the dogs in group 2 had pathological levels of D-dimer (>0.5 µg/mL). In group 3, 32.6% had pathological levels of D-dimer higher than 0.5 µg/mL. Negative troponin T levels appeared in the group of healthy animals, the amicrofilaremic group, and the microfilaremic group, which seems to indicate that the presence of heartworms in the right side of the heart is not associated with a significant myocardial cell injury when they do not cause any signs that point to right-sided heart failure.

Whether measurements of troponin T concentration will provide a reliable test method for assessing the degree of cardiac damage in symptomatic heartworm disease still remains to be proven in future studies. In group 2 and group 3, myoglobin concentrations were also abnormally high. However, myoglobin may come from either the heart or skeletal muscle, so an increase in serum myoglobin is not specific to damage to the heart. Consequently, these high serological levels do not provide any conclusive data. Our results suggest that in dogs infected with *Dirofilaria immitis,* biomarkers of pulmonary thromboembolism (D-dimer) were high, especially in microfilaremic dogs (group 3). Therefore, until further information on clinical patients is known, the D-dimer test appears to be a supportive test in the diagnosis of thromboembolism in dogs with *Dirofilaria immitis* and as a prognostic indicator of the disease. The results of this study are encouraging and justify a larger prospective study in order to evaluate and perhaps provide further evidence to support the use of cardiopulmonary biomarkers in diagnosis, prognosis, and management of heartworm disease encountered in veterinary medicine.

Research supported by Agencia Canaria de Investigación, Innovación y Sociedad de la Información, Gobierno de Canarias (Grant C20080100093).

1 Faculty of Veterinary, University of Las Palmas de Gran Canaria, 35307- Arucas (Las Palmas), Spain
2 Laboratory of Parasitology, Faculty of Pharmacy, University of Salamanca 37007 Salamanca, Spain

Novel Adulticide Therapy for *Dirofilaria immitis* Infection: A Clinical Case in a Naturally Infected Dog

G. Grandi,[1] A. Mavropoulou,[1] A. Volta,[1] C. Quintavalla,[1] G. Gnudi,[1] L.Venco,[2] L. Kramer[1]

3:35-3:45 pm • Saturday, April 17, 2010

Canine heartworm disease is caused by infection with *Dirofilaria immitis*, a filarial nematode that resides in the pulmonary arteries and occasionally in the right heart chambers of infected dogs. In dogs, the presence of adult worms causes chronic changes in the pulmonary vascular system (inflammation, endothelial proliferation with thickening of the vessel wall, etc) that lead eventually to pulmonary hypertension and, if not treated, to congestive heart failure. Treatment options for adulticide therapy include administration of the arsenical derivative melarsomine hydrochloride (Immiticide®, Merial), but side effects due to thromboembolism can be severe. Monthly administration of preventive doses of ivermectin have also been shown to be filaricidal, but dogs become negative for circulating *D. immitis* antigens (indication of complete elimination of the parasite population) only after 24-36 months. Like many filarial worms, *D. immitis* harbours the bacterial endosymbiont *Wolbachia*, which is essential for worm reproduction and long-term survival. Removal of the bacteria from the worm through antibiotic treatment of the infected host with tetracyclines can lead to worm death.

Here, the authors show the results of a combination of doxycycline (10 mg/kg sid for 30 days) and ivermectin (6 µg/kg/15 days for 6 months) in a naturally infected dog from Italy. The dog was evaluated once a month for 6 months and again 4 months later. Parameters evaluated at each visit included: microfilarial enumeration (modified Knott's test), testing for circulating *D. immitis* antigens (PetChek®, IDEXX Laboratories), thoracic radiography, and cardiac ultrasound. A Knott's test revealed the presence of 29,910 microfilariae/mL. One adult parasite was observed at echocardiographic examination, while radiographic examination showed the presence of typical heartworm-related alterations of moderate degree. The dog became negative for the presence of circulating microfilariae at day 60, and antigenemia became negative at day 120. Except for a slight and temporary worsening of echocardiographic parameters, none of the clinical parameters worsened during therapy (general condition, pulmonary vascular, bronchial and interstitial pattern as observed by radiography), the dog remained negative throughout the rest of the study period and is still completely cured at the 4-month follow-up. Moreover, at the last visit, no worms could be observed by echocardiography. The authors suggest that a combination of doxycycline and ivermectin (or another macrocyclic lactone) may be a valid alternative for adulticide therapy in *D. immitis* naturally infected dogs.

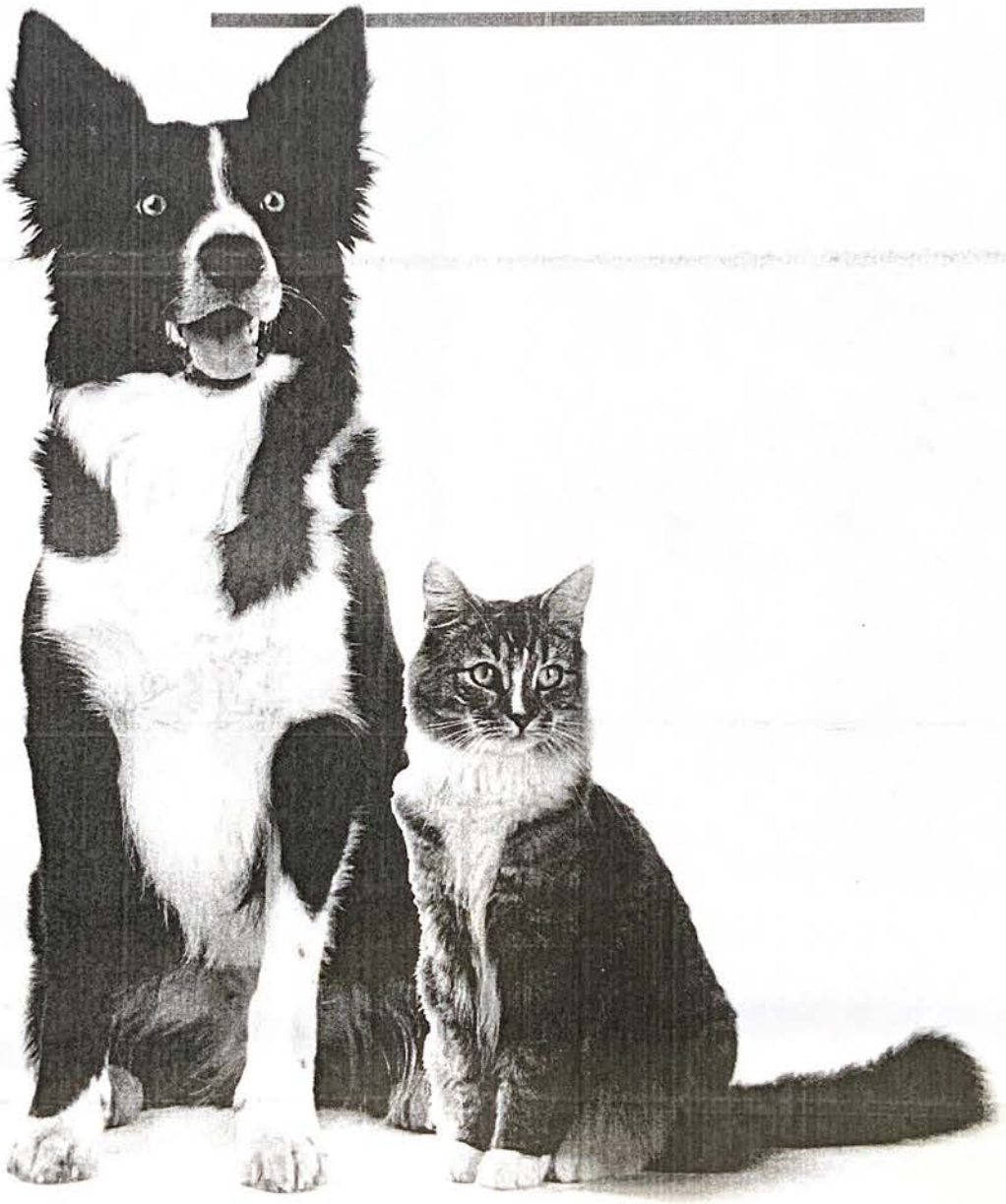
Acknowledgement: This study was funded by the project

STATE OF THE HEARTWORM SYMPOSIUM

2010

AMERICAN HEARTWORM SOCIETY

13th Triennial STATE OF THE HEARTWORM SYMPOSIUM



13th Triennial Symposium

■

April 16-18, 2010

■

The Peabody

■

Memphis
Tennessee

STATE OF THE HEARTWORM SYMPOSIUM

2010

AMERICAN HEARTWORM SOCIETY

The American Heartworm Society Extends a Warm Welcome to Those Attending the 13th Triennial Symposium in 2010

The Society was formed in 1974 to further scientific progress in the study of heartworm disease, to inform its membership of new developments, and to serve as a global resource to encourage and help promote effective procedures for the diagnosis, treatment, and prevention of heartworm disease.

www.heartwormsociety.org

CONTENTS

The American Heartworm Society State of the Heartworm Symposium 2010 meets the requirements for 15 hours of continuing education credit in jurisdictions that recognize the American Association of Veterinary State Boards Registry of Approved Continuing Education (AAVSB RACE) approval; however, participants should be aware that some boards have limitations on the number of hours accepted in certain categories and/or restrictions on certain methods of delivery of continuing education. AAVSB RACE program approval number: 521-6473.

AAVSB race