

Evaluation of a single injection of a sustained-release formulation of moxidectin for prevention of experimental heartworm infection after 12 months in dogs

Merete Holm-Martin, DVM, and Rick Atwell, BVSc, PhD

Objective—To evaluate the efficacy of a single injection of a sustained-release formulation of moxidectin in preventing heartworm (*Dirofilaria immitis*) infection for 12 months in dogs.

Animals—14 healthy dogs.

Procedure—Group A (nontreated control dogs; n = 6) received sterile vehicle administered SC, and group B (treated dogs; n = 6) received a sustained-release formulation of moxidectin administered SC. All dogs were housed in a heartworm-endemic area for 11.5 months, and heartworm antigen and modified Knott tests were performed monthly. All dogs (including 2 additional control dogs [group C]) were then inoculated with infective-stage larvae (L3) of *D immitis*, and 4.5 months later, all dogs were euthanatized and post-mortem examinations were performed. Adult *D immitis* were counted and measured, and their age was estimated.

Results—All dogs in groups A and C were infected with young (4- to 4.5-month old) adult male and female *D immitis*. No dogs in group B were infected with heartworms.

Conclusions and Clinical Relevance—The age of heartworms recovered suggests that infection was the result of experimental inoculation and not natural exposure to mosquitoes during the 11.5-month period the dogs resided in a heartworm-endemic area. A single SC injection of a sustained-release formulation of moxidectin was effective in providing protection against heartworm infection after 12 months in dogs. This formulation is a convenient method of heartworm prophylaxis that could eliminate the problem of poor owner compliance. (*Am J Vet Res* 2004; 65:1596–1599)

Dirofilaria immitis and its transmission and heartworm disease and its treatment and prevention have been studied extensively. *Dirofilaria immitis* is found in temperate and tropical coastal zones of the Atlantic, Pacific, and Indian Oceans and the

Mediterranean Sea, and heartworm disease is highly prevalent in the United States,^{1,2} Australia,³ and Japan.⁴ The transmission of *D immitis* depends on the availability of the vector and obligate intermediate host; many species of mosquito found throughout tropical and temperate climatic zones can transmit the infection.

Preventative agents that kill the tissue-migrating infective larvae of *D immitis* are used in endemic areas. Until recently, prevention of heartworm disease has relied on daily treatment of dogs with diethylcarbamazine citrate or monthly treatment with macrocyclic lactones. However, these dosing regimens are associated with problems of owner compliance, and late treatments and missed doses are common. A preventative medication that is administered once annually would eliminate the problem of owner compliance and could be included in routine vaccination and health check programs for dogs.

Moxidectin is a second-generation macrocyclic lactone in the milbemycin group. It is derived from the naturally occurring compound nemadectin and is used extensively as an anthelmintic and ectoparasiticide in cattle, sheep, and horses worldwide. Moxidectin for heartworm prevention was introduced in Australia in 1994. This product,^a a tablet for use in dogs, is administered once monthly (3 µg/kg, PO) and kills third stage larvae (L3) of *D immitis* for up to 2 months.^{5,6}

An injectable, sustained-release formulation of moxidectin has been evaluated. A single SC injection (0.5 mg of moxidectin/kg) resulted in a mean serum moxidectin concentration > 0.5 µg/mL for more than 10 months,^b indicating that 12 months' prevention of heartworm infection in dogs would be likely at this dose. The purpose of the study reported here was to evaluate the efficacy of a single injection of a sustained-release formulation of moxidectin in preventing heartworm infection for 12 months in dogs.

Materials and Methods

Dogs—Twelve mixed-breed dogs, 7 females and 5 males, were obtained for the commencement of the study (day 0). Two other dogs, 1 female and 1 male, were obtained just prior to the time of experimental inoculation with L3 of *D immitis*. All dogs originated from a heartworm-free area (Toowoomba) in Queensland, and all had negative results of tests for adult *D immitis* antigen.^c Dogs were 2 to 6 years of age and weighed 11.2 to 30.5 kg. All dogs were routinely vaccinated and treated for intestinal parasites with praziquantel, fenbantal, and pyrantel embonate before

Received August 18, 2003.

Accepted September 26, 2003.

From Fort Dodge Australia Pty Ltd, PO Box 6024, Baulkham Hills BC, NSW, 2153, Australia (Holm-Martin); and the Department of Companion Animal Medicine & Surgery, School of Veterinary Science, University of Queensland, Brisbane, Qld, 4072, Australia (Atwell).

Presented in abstract form at the Fort Dodge Animal Health Seminar held in conjunction with the 18th International Conference of the World Association for the Advancement of Veterinary Parasitology, Stresa, Italy, August 2001.

Address correspondence to Dr. Holm-Martin.

the study began. Sequentially numbered dogs were identified by sex, breed, and a detailed coat and color description. The study was approved by the University of Queensland Animal Ethics Committee.

Test substance—The sustained-release formulation of moxidectin for injection^d is a 10% suspension of microspheres (triglycerides) containing moxidectin in a sterile vehicle. A dose of 0.5 mg of moxidectin/kg, SC, was used.

Source of L3 for experimental inoculation—Laboratory-reared mosquitoes (*Aedes aegypti*) were infected with microfilariae from heartworm-infected dogs at the Army Malarial Research Unit at Enoggera, Australia. Two dogs with circulating microfilariae (confirmed on examination of a thick blood smear via light microscopy and a modified Knott test at the University of Queensland) were anesthetized with pentobarbitone and positioned in lateral recumbency. Mosquitoes were placed in feeding cups, and cups were placed on shaven skin of dogs for approximately 7 to 8 minutes. When most of the mosquitoes had fed, cups were removed and the mosquitoes were moved back to the insectary for 13 to 14 days. Prior to inoculation of the 14 dogs on days 368 and 369, mosquitoes were narcotized with CO₂ and the L3 were collected by use of the method described by Lok et al.⁷

Housing of dogs—The 12 dogs obtained for the commencement of the study were housed in a kennel in a heartworm-endemic area of northern Australia (Darwin) for approximately 11.5 months (day 0 to day 348). Each dog was housed individually in a run and had access to an exercise yard daily. Afterwards, dogs were moved to a kennel in Brisbane, Queensland, and kept in compatible pairs with access to exercise yards. Two dogs that entered the study on day 348 were housed at another dog kennel in Brisbane. At each facility, the dogs were fed commercial dog food and had access to water ad libitum.

Experimental design—On day 0, dogs were weighed, ranked in descending order of body weight, and allocated to blocks of 2 dogs each. Dogs from each block were randomly assigned to group A or B. Group A (nontreated control dogs) received an SC injection of sterile vehicle (0.05 mL/kg). Group B (treated dogs) received an SC injection of test substance (0.05 mL/kg [0.5 mg of moxidectin/kg]). Group C dogs joined the study as additional nontreated dogs on day 348. Group C dogs were used to verify the viability of the L3 challenge and to assist in the determination of time of infection of group A and B dogs.

Dogs were observed for adverse reactions for 6 hours after treatment, twice daily on days 1 to 7, and daily to day 14. Injection sites were palpated daily on days 1 to 14, weekly for the subsequent 4 weeks, and every 2 weeks until dogs were transported to Brisbane on day 348. Blood samples were collected every 4 weeks, and adult *D immitis* antigen tests^e and modified Knott tests were performed to assess heartworm status.

On day 369, group C dogs were each experimentally inoculated with 20 L3 of *D immitis*. On days 368

or 369, dogs in group A were each experimentally inoculated with 20 L3. On days 368 or 369, 4 dogs in group B were inoculated with 20 L3 and 2 dogs were inoculated with 8 and 7 L3, respectively. Unexpectedly high mosquito mortality rate resulted in collection of less L3 than planned. The available L3 were divided into as many doses as possible of 20 L3, and the remainder was divided into 2 doses of 8 and 7 L3, respectively. The allocation of L3 was blinded and dogs were randomly selected for inoculation. Larvae were injected SC in the lower left neck area by use of an 18-gauge needle and a 1-mL insulin syringe.⁷

At 130 and 133 days after inoculation with L3 (days 498 and 502, respectively), all dogs were euthanized with an overdose of pentobarbital sodium administered IV and postmortem examinations were performed. The pleural and abdominal cavities and venae cavae were examined for heartworms. The heart and lungs were removed from the thoracic cavity, and the pulmonary artery, right ventricle, right atrium, and venae cavae were opened in sequence and inspected. The pulmonary artery was dissected along each of its 7 main branches to approximately the 1-mm-diameter level. Lesions and numbers of heartworms found were recorded. The sex⁸ and size^{8,9} of each heartworm were determined, and size data were used to approximate the time of infection (ie, natural infection in Darwin vs experimental inoculation).

On the basis of number of L3 inoculated, percentage recovery of *D immitis* was calculated:

$$\text{Percentage recovery} = \frac{\text{Number of adults recovered}}{\text{Number of L3 inoculated}} \times 100.$$

Statistical analyses—Adult heartworm counts in group B dogs were compared with those of group A dogs by use of 1-tailed Student *t* test. Values of *P* < 0.05 were considered significant.

Results

Heartworm tests—No dogs had positive test results for the modified Knott test or adult *D immitis* antigen test before commencement of the study or during the study.

Postmortem examination—All dogs in groups A and C were infected with adult male and female *D immitis* estimated to be 4 to 4.5 months old.⁸ No heartworms were found in dogs in group B (Table 1). No macroscopic lesions other than mild pulmonary vasculitis (a result of dirofilariasis) were detected in other organs of all dogs in groups A and C. On the basis of the number of L3 inoculated, adult recovery rate was 38.3% in group A and 42.5% in group C.

Injection site reactions—A 5-mm-diameter swelling at the injection site was palpated on day 6 in 1 dog in group B. On days 7 to 10, the swelling was barely visible, firm, and approximately the same size. Between days 11 and 17, the swelling was not visible but was palpable as a soft lesion 5 mm in diameter. By days 18 and 19, the swelling was smaller, and by day 20, it was undetectable. No other dogs developed injection site lesions. Postmortem examination of injection sites

Table 1—Number of infective-stage larvae (L3) of *Dirofilaria immitis* inoculated and number of adult *D immitis* recovered at postmortem examination in 3 groups of dogs.

Group	No. L3 Inoculated	No. adult males	No. adult females	No. adults	Recovery (%)
Group A (n = 6)	120	19	27	46	38.3
Group B (6)	95	0	0	0	0
Group C (2)	40	9	8	17	42.5

Group A = Nontreated control dogs. Group B = Dogs treated with a sustained-release formulation of moxidectin. Group C = Additional nontreated control group.

approximately 16.5 months after treatment revealed no macroscopic subcutaneous tissue changes. No adverse effects were associated with injection of the test substance. No adverse clinical reactions were observed in any of the dogs throughout the study period.

Concurrent treatments—During the 502-day study period, standard veterinary procedures were followed to deal with a variety of medical conditions. Dogs were treated for problems that included infectious tracheobronchitis, otitis externa, gastrointestinal disturbance, and dental plaque.

Adult heartworm counts—Seven, 9, 7, 10, 8, and 5 adult *D immitis* were found in each of 6 dogs in group A, and no adult *D immitis* were found in any dogs in group B; this difference was significant ($P = 0.001$). The power of the test was 77%. Eight and 9 adult *D immitis* were found in the 2 dogs in group C.

Discussion

Absence of mature adult heartworms (older than 4 to 4.5 months) suggests that infection via natural exposure to mosquitoes was unsuccessful during the 11.5-month period dogs were housed in Darwin. This finding is supported by negative results of modified Knott tests and *D immitis* antigen tests in all dogs during this period. The lack of heartworm infection of dogs residing in a heartworm-endemic area for 11.5 months may have been the result of a very prolonged dry season during the year of the study, which affected the population of mosquitoes.¹⁰⁻¹² The unusual dryness of an area that is normally affected by monsoons would result in lack of surface water, and therefore a decrease in the breeding sites available for water-breeding insects. This would result in a decreased mosquito larval survival rate and adult mosquito emergence rate.

The unexpected high mortality rate (> 80%) of laboratory-reared mosquitoes and the low number of L3 of *D immitis* harvested resulted in a reduced number of L3 available for inoculation. This resulted in a reduced challenge of 20 L3/dog for all dogs in groups A and C and for 4 dogs in group B. The remaining 2 dogs in group B were challenged with only 7 and 8 L3, respectively. The group challenge, however, was still 95 L3 for group B versus 120 L3 for group A, and a mean recovery rate of 40% was achieved. We initially planned to inoculate 50 L3/dog, as was used in similar studies.^{3,7b}

According to the experience of the second author, infection does not seem to depend on the number of L3 inoculated, and in our study, infection was achieved in all control dogs in groups A and C. Treatment with moxidectin appeared to be as effective after inoculation of 20 L3 as after inoculation of 7 and 8 L3.

After experimental inoculation of L3, dogs treated with a single injection of a sustained-release formulation of moxidectin were protected against heartworm infection, whereas nontreated control dogs were not. The number of dogs in each group was sufficient to detect any significant differences.

The initial intent of evaluating the efficacy of a sustained-release formulation of moxidectin in preventing heartworm infection for 12 months was not possible because of the lack of natural challenge of dogs during their stay in a heartworm-endemic area; however, the sustained-release formulation of moxidectin protected against heartworm infection after experimental inoculation of L3 after 12 months. Field use of the commercial product in Australia for 4 years (> 1.5 million doses administered) has not resulted in known reports of heartworm infection; this data¹³ supports our initial hypothesis.

An injectable, sustained-release formulation of moxidectin provided protection against heartworm infection after 1 year and was not associated with adverse effects. The sustained-release formulation is a convenient method of heartworm prophylaxis that could eliminate the problem of poor owner compliance.

^aProHeart Tablets for dogs, Fort Dodge Australia Pty Ltd, Sydney, NSW, Australia.

^bFort Dodge Animal Health. *Prophylactic activity of moxidectin SR injectable formulation against Dirofilaria immitis infections in beagles*, 1998. GASD 05-14.00. Sydney, Australia: Fort Dodge Animal Health, 1998. Available on request from Fort Dodge Australia Pty Ltd, Sydney, NSW, Australia.

^cWitness, Agen Biomedical, Acacia Ridge, Queensland, Australia.

^dProHeart SR-12 Injection Once-a-Year Heartworm Preventative for Dogs, Fort Dodge Australia Pty Ltd, Sydney, NSW, Australia.

^eDiroCHECK, Agen Biomedical, Acacia Ridge, Queensland, Australia.

References

- Otto GF. Changing geographic distribution of heartworm disease in the United States, in *Proceedings*. 1st Triennial Symp Am Heartworm Soc 1974;1-2.
- Walters LL. Risk factors for heartworm infection in Northern California, in *Proceedings*. 8th Triennial Symp Am Heartworm Soc 1995;5-26.
- Carlisle CH, Atwell RB. A survey of heartworm in dogs in Australia. *Aust Vet J* 1984;61:356-360.
- Pacheco G. Synopsis of Dr. Seiji Kume's reports at the first international symposium on canine heartworm disease, in *Proceedings*. 1st Triennial Symp Am Heartworm Soc 1974;137-139.
- McCall JW, McTier TL, Holmes RA, et al. Prevention of naturally acquired heartworm infection in heartworm-naive beagles by oral administration of moxidectin at an interval of either one or two months, in *Proceedings*. 7th Triennial Symp Am Heartworm Soc 1992;169-177.
- McTier TL, McCall JW, Dzimiński MT, et al. Prevention of experimental heartworm infection in dogs with single oral doses of moxidectin, in *Proceedings*. 7th Triennial Symp Am Heartworm Soc 1992; 165-168.

7. Lok JB, Knight DH, Wang GT, et al. Activity of an injectable, sustained-release formulation of moxidectin administered prophylactically to mixed-breed dogs to prevent infection with *Dirofilaria immitis*. *Am J Vet Res* 2001;62:1721-1726.
8. Orihel TC. Morphology of the larval stages of *Dirofilaria immitis* in the dog. *J Parasitol* 1961;47:251-262.
9. Lok JB. *Dirofilaria* sp: taxonomy and distribution. In: Boreham PFL, Atwell RB, eds. *Dirofilariasis*. Boca Raton, Fla: CRC Press Inc, 1988; 1-28.
10. Whelan PI, Jacups SP, Melville L, et al. Rainfall and vector mosquito numbers as risk indicators for mosquito-borne disease in central Australia. *Commun Dis Intell* 2003;27:110-116.
11. Russell RC. Mosquito-borne arboviruses in Australia: the current scene and implications of climate change for human health. *Int J Parasitol* 1998;28:955-969.
12. Kelly-Hope LA, Purdie DM, Kay BH. Ross River virus disease in Australia, 1986-1998, with analysis of risk factors associated with outbreaks. *J Med Entomol* 2004;41:133-150.
13. Zwijnenberg R. Fort Dodge Pty. Limited Adverse Reaction Database; Accessed 2002.



Correction: Distribution of mRNA that codes for 5-hydroxytryptamine receptor subtypes in the gastrointestinal tract of dairy cows

In the article "Distribution of mRNA that codes for 5-hydroxytryptamine receptor subtypes in the gastrointestinal tract of dairy cows," published August 2004 (2004;65:1151-1158), the following items were incorrect.

In Table 2 on page 1154, the letters for the location colon A were incorrectly listed as A-D and d for column 5-HTR1B and A, E, F, and G for column 5-HTR1D. The correct letters are A-D for column 5-HTR1B and A, E, F, G, and d for column 5-HTR1D.