

Participation by Academia in Discovery and Development of Products by the Animal Health Industry

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The complex process of developing health-related products for use in animals requires a substantial commitment to basic research by industry. While most companies maintain a reasonable number of highly competent, and internationally recognized, research scientists, it is virtually impossible for industry to permanently staff all of the highly specialized individuals that are needed for discovery and development of their wide range of products. This is particularly true today when management and competitive marketing strategies demand a product or combination of products that achieve a broad spectrum of activity. So, rather than redirect its current staff or hire additional personnel, industry frequently exercises its option to search outside of its corporate infrastructure for expertise in specific subjects. Universities and small, highly specialized private research enterprises are unique sources of such expertise. As much by necessity as by choice, the researcher in academia tends to be highly specialized and to work independently. Unfortunately, this tends to limit the individual's ability to maintain sufficient financial support to sustain a productive research program. There are very few granting agencies (e.g., government agencies, private foundations) that fund basic research in many important areas of animal health. Moreover, the growing trend toward the funding of solicited, goal-oriented proposals, some of which are earmarked for "politically correct" projects, further limits the sources of financial support for research in these areas.

While most of the research conducted by and for industry is professionally stimulating and attractive to researchers in academia and industry, some of it is considered pedestrian, particularly by university administrators, and thus is often shunned by academic researchers. Frequently, this obstacle can be overcome by adding nominal amounts of extra funds to the somewhat mundane project to allow the investigator to pursue more academically appealing research projects. Often, the data from such research projects can be utilized by the company (sponsor) in future, as well as current, product development.

In view of this, the collaboration of academia and goal-oriented, market-driven industry has been and should continue to be mutually beneficial. This presentation is to describe how industry itself, my research programs, and the scientific community in general have benefited from industry-supported, ancillary research on heartworm disease conducted in my laboratories during the past several years. While this research ultimately benefits industry, it is not conducted as part of a GCP or GLP protocol issued by a sponsor. Rather, this research represents the creative thoughts and initiative provided by scientists in academia. I will illustrate this through independent research my laboratory group has conducted, without specific sponsorship by industry.

For convenience, I have arbitrarily categorized this research as (1) need-driven, which includes primarily the development, optimization, and/or standardization of models for specific types

of heartworm studies in the laboratory; (2) market-expansion, which deals mainly with modifying the "label" usage or widening the spectrum of activity of existing commercial products; and (3) gap-filling, which deals with further definition of the accuracy, limitations, and overall usefulness of commercially available products.

Need-Driven Research

The development and optimization of methods for cyclical maintenance of several filarial parasites in their vertebrate and invertebrate hosts (McCall, 1981) has provided a "core" of research materials for our studies on animal models for research in filariasis, particularly heartworm (*Dirofilaria immitis*) disease. We have developed and standardized heartworm infections induced by subcutaneous (SC) inoculation of third-stage infective larvae (L₃) in dogs (Dzimianski *et al.*, 1989b), cats (McTier *et al.*, 1992b), and ferrets (Supakorndej *et al.*, 1992, 1994); by IV transplantation of adult worms in dogs (Dzimianski *et al.*, 1989b) and cats (McCall *et al.*, 1992); and by SC transplantation of 3- to 4.5-month-old worms into ferrets (Tanner, 1984). Selected parasitological data on these models is presented in Table 1. These models were developed mainly to enable us to conduct controlled trials to study heartworm disease in general and therapeutic intervention in particular. Among other studies, SC-induced infections have been used for evaluating the prophylactic activity of (1) ivermectin in dogs (McCall *et al.*, 1981), cats (McTier *et al.*, 1992b; Paul *et al.*, 1992), and ferrets (Supakorndej *et al.*, 1992); (2) milbemycin oxime in dogs (Blagburn *et al.*, 1989; Bradley, 1989; Grieve *et al.*, 1989) and cats (Stewart *et al.*, 1992); (3) moxidectin in dogs (McTier *et al.*, 1992a); and (4) diethylcarbamazine citrate with and without oxibendazole (McCall *et al.*, 1987) in dogs. Blair and co-workers (1983) used SC-induced infections to study the efficacy of thiacetarsamide against larval and adult heartworms, and Dzimianski and co-workers used such infections to show the

clinical prophylactic activity of RM 340 (a new arsenical adulticide) against 4-month-old heartworms. Infections of IV-transplanted adult worms have been used for various types of chemotherapeutic studies, for example, adulticidal activity of thiacetarsamide (Holmes *et al.*, 1986; Dzimianski *et al.*, 1989b) and RM 340 (Dzimianski *et al.*, 1989b; Keister *et al.*, 1992) in dogs, and microfilaricidal activity of ivermectin in cats (Alva *et al.*, 1993) and dogs (McCall, unpublished data).

Market-Expansion Research

For several years, we suspected that the prophylactic dosage of ivermectin was efficacious against immature heartworms in the heart and lungs as well as against 1- to 2-month-old larvae. So, selecting the 4-month-old worm as the main target, we conducted a pilot experiment with a limited number of dogs to examine whether macrolides had clinical prophylactic activity against *D. immitis* (McCall *et al.*, 1993a). This was an unapproved use which was not supported by the sponsor. Three groups of 3 heartworm-naive beagles were inoculated SC with *D. immitis* L₃ (50/dog). One group was given ivermectin (Heartgard-30®, Merck & Co.) *per os* monthly at 6.0 mcg/kg for 13 months (14 doses) beginning at 4 months postinoculation (PI); another group was given milbemycin oxime (Interceptor®, CIBA) *per os* monthly at 500 mcg/kg during the second period; another group served as the nontreated control. None of the ivermectin-treated dogs ever had circulating microfilariae; only 2 of the milbemycin-treated dogs had a microfilaremia (1 dog had 2/ml at 8 months PI and the other had 9/ml at 9 months PI). All of the control dogs developed high microfilaremias. Adult heartworm antigen (DiroCHEK®, Synbiotics Corp.) test scores were consistently high in both the milbemycin-treated dogs and the controls (Table 2). In contrast, one of the 3 ivermectin-treated dogs was antigen-positive through necropsy, but the

remaining 2 dogs had transient antigenemias during the study. At necropsy (18 months PI), 1 month after the last monthly treatment, ivermectin proved to be 98% effective in suppressing infection, while milbemycin was only 49% effective (Table 3). All of the control dogs had heartworms (geo. mean, 27.0/dog).

This experiment has now received industry's attention and is being repeated using more (5) dogs per group, and 2 groups treated with ivermectin and milbemycin, respectively, beginning at 3 months PI have been added. At this time (13 months PI), the microfilaremia and antigenemia patterns in the dogs treated beginning at 4 months PI are similar to those seen in the pilot experiment. The absence of microfilariae and detectable antigen in dogs treated with ivermectin and milbemycin beginning at 3 months PI strongly suggest that this schedule is even more efficacious. The dogs will be necropsied at 17 to 18 months PI. The accuracy of the antigen test in predicting the clinical prophylactic activity of ivermectin, and the lack thereof with milbemycin, is encouraging.

These trials were prompted by our desire to understand the basic research question regarding the efficacy of avermectins against young adult heartworms, rather than by industry's recognition of the potential for development of a new claim(s).

Gap-Filling Research

With the above demonstration that monthly prophylactic treatment with milbemycin induces occult infections (i.e., amicrofilaremic infections with adult heartworms) and the recent report that monthly administration of prophylactic doses of ivermectin or milbemycin to microfilaremic dogs leads to occult infections within 6 to 9 months (Bowman *et al.*, 1992), the role of antigen tests in diagnosing infection has become more important. Fortunately, no more than 1% of the microfilaremic dogs are not positive on an antigen test. So, the American

Heartworm Society (1992) now recommends that antigen tests be used both for routine survey of dogs in heartworm endemic areas and for confirmation of suspected cases.

In general, heartworm antigen tests have been considered to be highly specific and sensitive, detecting most infections with 3 or more female worms at least 8 months of age, and they have not been considered to be effective in detecting infections with worms less than about 5 months of age (Dzimianski & McCall, 1986; Dzimianski *et al.*, 1989a). Male worms were reported to contribute substantially to the antigen level (Courtney *et al.*, 1990; Ely & Courtney, 1987), with as many as 70% of the dogs with only male worms being detected (Courtney *et al.*, 1988). Gaps in our knowledge of the effectiveness and limitations of these tests greatly reduced our confidence in interpreting both positive and negative test results. Fortunately, with my laboratory's sustained research program in heartworm disease, we were in a position to address these questions.

The most definitive way to fill these gaps was to further define the utility of individual test kits and to compare the sensitivity of several kits using experimentally induced low-level infections established by IV transplantation of known numbers of male and/or female worms into beagles (McCall *et al.*, 1994). The use of such infections in evaluating antigen diagnostic tests greatly reduces the variables unpredictably encountered using naturally infected, random-source dogs.

In the first experiment, the accuracy of ELISA-based adult heartworm antigen tests in detecting single-sex infections of low to moderate intensity was examined using 21 heartworm-naive beagles given 16-month-old *D. immitis* by IV transplantation (McCall *et al.*, 1993b). Sensitivity of these kits (ASSURE®/CH, DiroCHEK®, UNI-TEC®/CHW, Synbiotics Corp., San Diego, CA; CITE® Semi-Quant™, Snap™, PetChek®, IDEXX Corp., Portland, ME) was compared using 3 groups of 4 dogs each with 1, 2, or 3 female worms only, respectively; 1 group of

4 dogs with 5 male worms only; 1 group of 2 dogs with 10 male worms only; and 1 group of 3 dogs with 13 male worms only. At 10 to 17 weeks posttransplantation (PTR), all of the test kits detected antigen in all of the dogs with female worms, even those with only a single female worm. In addition, the rate at which antigen appeared was examined by testing at 1, 2, 4, and 10 weeks PTR in dogs given 1, 2, or 3 female worms using the DiroCHEK® and CITE® Semi-Quant™ kits. With both kits, the rate at which detectable antigen appeared after transplantation depended on the number of female worms present, with infections of 3 worms being detected the earliest; earlier detection of these infections by the DiroCHEK® test indicated that it was more sensitive than the CITE® Semi-Quant™ test. To examine whether this detectable level of antigen also was produced by younger, virgin female worms; to confirm the lack, or low level, of antigen produced by male worms; and to monitor seroconversion in early infections, we undertook a two-part experiment with a total of 24 beagles. Virgin female and/or male heartworms 113 to 116 days old were transplanted. Groups of 4 dogs each were given 1, 2, 3, or 7 single-sex infections of female worms only; 1 group of 4 dogs was given 3 female and 3 male worms; and 2 groups of 2 dogs each were given single-sex infections of 13 and 24 male worms only, respectively. As summarized in Table 4, infections with female worms less than 6 months of age were not detected regardless of the number of worms; thereafter, the number of female worms influenced the youngest age at which all of the infected dogs in a group could be detected, with those with the most worms being detected earliest and those with the fewest worms detected the latest. Although the test reactions were weak to low, all of the 3 dogs that had a single live female worm were positive by the time they were 7.5 months old. As in the previous study, none of the dogs with only male worms were antigen-positive. The absence of circulating microfilariae throughout the study in all of the dogs given single-sex infections indicated that

mating had not occurred prior to transplantation. Similar antigenemias in the dogs with 3 female worms only and those with 3 males and 3 females indicated that the reproductive status of female worms has little, if any, influence on the antigen level.

Conclusion

The data presented here largely represent selected examples of ancillary research projects conducted in my laboratories using unexpended balances of funds from several industry-supported "discovery" (and "development") projects. These and other projects have enabled me to strengthen my basic research programs and, at the same time, have provided useful information to the animal health industry for potential new product development, redefinition of current product profiles, and market expansion of existing products.

References

- Alva, R., Dzimianski, M.T., McTier, T.L. & McCall, J.W. (1993) Safety of ivermectin in cats with patent infections of *Dirofilaria immitis*. (Abstract) In *Proceedings of the 38th Annual Meeting of the American Association of Veterinary Parasitologists*. pp. 49, Minneapolis, Minnesota.
- American Heartworm Society. (1992) Recommended procedures for the diagnosis and management of heartworm (*Dirofilaria immitis*) infection. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 289-294. American Heartworm Society, Batavia, Illinois.
- Blagburn, B.L., Hendrix, C.M., Lindsay, D.S., Vaughan, J.L., Mysinger, R.H. & Hepler, D.I. (1989) Milbemycin: Efficacy and toxicity in beagle and collie dogs. In *Proceedings of the Heartworm Symposium '89*. Ed Otto, G.F. pp. 109-113. American Heartworm Society, Washington, DC.
- Blair, L.S., Malatesta, P.F., Gerckens, L.S. & Ewanciw, D.V. (1983) Efficacy of thiacetarsamide in experimentally infected dogs at 2, 4, 6, 12, or 24 months postinfection with

Dirofilaria immitis. In *Proceedings of the Heartworm Symposium '83*. Ed Otto, G.F. pp. 130-133. Veterinary Medicine Publishing Co., Edwardsville, Kansas.

Bowman, D.D., Johnson, R.B., Ulrich, M.E., Neumann, N., Lok, J.B., Zhang, Y. & Knight, D.H. (1992) Effects of long-term administration of ivermectin or milbemycin oxime on circulating microfilariae and parasite antigenemia in dogs with patent heartworm infections. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 151-158. American Heartworm Society, Batavia, Illinois.

Bradley, R.E. (1989) Dose titration and efficacy of milbemycin oxime for prophylaxis against *Dirofilaria immitis* infection in dogs. In *Proceedings of the Heartworm Symposium '89*. Ed Otto, G.F. pp. 115-120. American Heartworm Society, Washington, DC.

Courtney, C.H., Zeng, Q.Y. & Bean, E.S. (1988) Sensitivity and specificity of the DiroCHEK® heartworm antigen test for immunodiagnosis of canine dirofilariasis and a comparison with other immunodiagnostic tests. *Journal of the American Animal Hospital Association*, **24**, 27-32.

Courtney, C.H., Zeng, Q.Y. & Tonelli, Q. (1990) Sensitivity and specificity of the CITE® heartworm antigen test and a comparison with the DiroCHEK® heartworm antigen test. *Journal of the American Animal Hospital Association*, **26**, 623-628.

Dzimianski, M.T. & McCall, J.W. (1986) Evaluation of adult antigen diagnostic test kits using well-defined dog sera from laboratory and field trials. In *Proceedings of the Heartworm Symposium '86*. Ed Otto, G.F. pp. 83-86. American Heartworm Society, Washington, DC.

Dzimianski, M.T., McTier, T.L. & McCall, J.W. (1989a) Evaluation of two adult heartworm antigen diagnostic test kits using well-defined dog and cat sera. (Abstract) In *Proceedings of the 34th Annual Meeting of the American Association of Veterinary Parasitologists*. pp. 33, Orlando, Florida.

Dzimianski, M.T., McTier, T.L., McCall, J.W. & Raynaud, J.P. (1989b) Assessment of filaricidal activity of a new filaricide (RM 340) against immature and adult heartworms using experimental canine models. In *Proceedings of the Heartworm Symposium '89*. Ed Otto, G.F.

pp. 147-153. American Heartworm Society, Washington, DC.

Ely, M.L. & Courtney, C.H. (1987) Sensitivity and specificity of Filarocheck® heartworm antigen test and Dirotect® heartworm antibody test for immunodiagnosis of canine dirofilariasis. *Journal of the American Animal Hospital Association*, **23**, 367-371.

Grieve, R.B., Frank, G.R., Stewart, V.A., Parsons, J.C., Abraham, D., MacWilliams, P.S. & Hepler, D.I. (1989) Effect of dosage and dose timing on heartworm (*Dirofilaria immitis*) chemoprophylaxis with milbemycin. In *Proceedings of the Heartworm Symposium '89*. Ed Otto, G.F. pp. 121-124. American Heartworm Society, Washington, DC.

Holmes, R.A., McCall, J.W., Prasse, K.W. & Wilson, R.C. (1986) Thiacetarsamide sodium: Pharmacokinetics and the effects of decreased liver function on efficacy against *Dirofilaria immitis* in dogs. In *Proceedings of the Heartworm Symposium '86*. Ed Otto, G.F. pp. 57-63. American Heartworm Society, Washington, DC.

Keister, D.M., Dzimianski, M.T., McTier, T.L., McCall, J.W. & Brown, J. (1992) Dose selection and confirmation of RM 340, a new filaricide for the treatment of dogs with immature and mature *Dirofilaria immitis*. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 225-229. American Heartworm Society, Batavia, Illinois.

McCall, J.W. (1981) The role of arthropods in the development of animal models for filariasis research. *Journal of Georgia Entomological Society*, **16**, 283-293.

McCall, J.W., Dzimianski, M.T., McTier, T.L., Jernigan, A.D., Jun, J.J., Mansour, A.E., Supakorndej, P., Plue, R.E., Clark, J.N., Wallace, D.H. & Lewis, R.E. (1992) Biology of experimental heartworm infections in cats. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 71-79. American Heartworm Society, Batavia, Illinois.

McCall, J.W., Evans, T.L., Lindemann, B.A., Murphy, J.M. & Simpson, J.E. (1987) Chemoprophylaxis of experimentally induced heartworm and hookworm infections in dogs. *Modern Veterinary Practice*, **July/August**, 417-421.

- McCall, J.W., Lindemann, B.A. & Porter, C.A. (1981) Prophylactic activity of avermectins against experimentally induced *Dirofilaria immitis* in dogs. In *Proceedings of the Heartworm Symposium '80*. Ed Otto, G.F. pp. 126-130. Veterinary Medicine Publishing Co., Edwardsville, Kansas.
- McCall, J.W., McTier, T.L., Supakorndej, N. & Ricketts, R.P. (1993a) Clinical prophylaxis of experimentally induced infections of *Dirofilaria immitis* by monthly treatment with Heartgard 30® beginning at four months PI. (Abstract) In *Proceedings of the 38th Annual Meeting of the American Association of Veterinary Parasitologists*. pp. 51, Minneapolis, Minnesota.
- McCall, J.W., McTier, T.L., Supakorndej, N., Ricketts, R. & Dzimianski, M.T. (1994) Further characterization of the sensitivity of several commercially available heartworm antigen test kits. In *Proceedings of The 1994 North American Veterinary Conference*. pp. 461-463, Orlando, Florida.
- McCall, J.W., Supakorndej, N., McTier, T.L., Dzimianski, M.T. & Ricketts, R.P. (1993b) Commercial heartworm antigen test kits detect infections with a single adult female worm but not with numerous adult male worms only. (Abstract) In *Proceedings of the 38th Annual Meeting of the American Association of Veterinary Parasitologists*. pp. 36, Minneapolis, Minnesota.
- McTier, T.L., McCall, J.W., Dzimianski, M.T., Aguilar, R. & Wood, I. (1992) Prevention of experimental heartworm infection in dogs with single, oral doses of moxidectin. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 165-168. American Heartworm Society, Batavia, Illinois.
- McTier, T.L., McCall, J.W., Dzimianski, M.T., Mansour, A.E., Jernigan, A., Clark, J.N., Plue, R.E. & Daurio, C.P. (1992) Prevention of heartworm infection in cats by treatment with ivermectin at one month postinfection. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 111-116. American Heartworm Society, Batavia, Illinois.
- Paul, A.J., Acre, K.E., Todd, K.S., Wallace, D.H., Jernigan, A.D. & Wallig, M.A. (1992) Efficacy of ivermectin against *Dirofilaria immitis* in cats 30 and 45 days postinfection. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 117-119. American Heartworm Society, Batavia, Illinois.
- Stewart, A.V., Blagburn, B.L., Hendrix, C.M., Hepler, D.I. & Grieve, R.B. (1992) Milbemycin oxime as an effective preventative of heartworm (*Dirofilaria immitis*) infection in cats. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 127-131. American Heartworm Society, Batavia, Illinois.
- Supakorndej, P., Jun, J.J. & McCall, J.W. (1994) Early migration and development of *Dirofilaria immitis* in the ferret, *Mustela putorius furo*. *Journal of Parasitology*, in press.
- Supakorndej, P., McCall, J.W., Lewis, R.E., Rowan, S.J., Mansour, A.E. & Holmes, R.A. (1992) Biology, diagnosis, and prevention of heartworm infection in ferrets. In *Proceedings of the Heartworm Symposium '92*. Ed Soll, M.D. pp. 59-69. American Heartworm Society, Batavia, Illinois.
- Tanner, P.A. (1984) Standardization of infection of *Dirofilaria immitis* in the ferret, *Mustela putorius furo*, by transplantation of immature worms. Master's thesis, The University of Georgia, Athens.

Table 1. Selected parasitologic data on various heartworm models.

Host	n	Parasite			Overall avg. % recovery (range)	Infection rate (%)	Selected reference
		Stage	No.	Route			
Dog ^a	47	L ₃	50	SC	56 (26-84)	100	Dzimianski <i>et al.</i> , 1989b
	39	Adult	5-15 pairs (M & F)	IV transpl.	91 (86-94)	100	Dzimianski <i>et al.</i> , 1989b
	18	Adult	1-3 F, 5-10 M	IV transpl.	100	100	McCall <i>et al.</i> , 1992
	24	Immature (113-116 days old)	1-7 F, 13-24 M	IV transpl.	92 (0-100)	96	McCall <i>et al.</i> , 1994
Cat ^b	26	L ₃	100	SC	6 (1-31)	73	McTier <i>et al.</i> , 1992b
	10	Adult	3-4 pairs (M & F)	IV transpl.	96 (83-100)	100	McCall <i>et al.</i> , 1992
Ferret ^b	9	L ₃	15	SC	53 (33-80)	100	Supakorndej <i>et al.</i> , 1992
	8	Immature (97-107 days old)	3 pairs (M & F)	SC transpl.	83 (50-100)	100	Tanner, 1984
	10	IMMature (117-137 days old)	3 pairs (M & F)	SC transpl.	35 (0-67)	90	Tanner, 1984

^a Microfilaremiias high and persistent.

^b Microfilaremiias low and transient.

Table 2. Summary of heartworm antigen test results in dogs (3/gp) treated monthly (x 14 doses) with ivermectin and milbemycin oxime beginning 4 months PI of *D. immitis* L₃.

Group	No. dogs ASSURE®/CH test positive at month PI											
	6 ^a	8	9	10	11	12	13	14	15	16	17	18
Control	ND ^b	3	ND	3	3	3	3	3	3	3	3	3
Ivermectin (6 mcg/kg)	0	3	2	2	2	2	2	2	1	1	1	1
Milbemycin (500 mcg/kg)	2	3	3	3	3	3	3	3	3	3	3	3

^a All dogs were antigen-negative prior to infection.

^b ND = not done

Table 3. Summary of live worm recovery and efficacy in dogs (3/gp) treated monthly (x 14 doses) with ivermectin and milbemycin oxime beginning 4 months PI of 50 *D. immitis* L₃.

Group	Live worm recovery geometric mean (range)*	Percent suppression
Control	27.0 (20-34)	NA
Ivermectin (6 mcg/kg)	0.6 (0-1)	98
Milbemycin (500 mcg/kg)	13.7 (10-25)	49

* Necropsy at 18 months.

Table 4. Summary of DiroCHEK® test results in beagles given virgin (113-116 days old) *D. immitis* by IV transplantation.

No. dogs	No. & sex of worms		Number of dogs antigen test positive by age of worms in months						
	Trans-planted	Re-covered	6.0 ^a	6.5	7.0	7.5	8.0	9.0	9.5-10.0
3 ^b	1F	1F	0	1	1	3	3	3	3
4	2F	2F	3	4	4	ND ^c	4	ND	4
4	3F	2-3F	3	4	3	4	4	4	4
4	3M, 3F	2-3M, 2-3F	3	3	4	4	4	4	4
4	7F	5-7F	4	4	4	ND	4	ND	4
2	13M	11-13M	0	0	0	0	0	0	ND
2	24M	23-24M	0	0	0	0	0	0	ND

^a All antigen-negative at 4.0 and 5.0 months

^b 1 additional dog was antigen-negative throughout the study; no live or dead worms or worm fragments were recovered at necropsy; data not included.

^c ND = not done