

# Evaluation of a covered-rod silicone implant containing ivermectin for long-term prevention of heartworm infection in dogs

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**Objective**—To evaluate use of covered-rod (CR) silicone implants containing ivermectin for long-term prevention of infection with *Dirofilaria immitis* in dogs.

**Animals**—145 adult male and female dogs.

**Procedures**—Dogs received implants of different sizes, and ivermectin concentrations and serum ivermectin concentrations were monitored for 16, 57, and 56 weeks, respectively, in 3 preclinical dose selection studies. Ability of implants to prevent infection with *D immitis* was evaluated in 2 further studies; dogs were challenged with 50 infective third-stage larvae 52 weeks after implant administration and necropsied 145 days after challenge, and the total number of adult heartworms was counted. A field study was then undertaken in which client-owned dogs received an implant and plasma samples were collected at intervals until week 52 for ivermectin analysis and heartworm antigen determination.

**Results**—Use of the implants resulted in maintenance of an ivermectin concentration  $\geq 0.2$  ng/mL for 12 months. In challenge studies, no treated dogs had adult heartworms, in contrast to untreated dogs, which all had adult heartworms at necropsy. In the field study, dogs treated with an implant had negative results of heartworm antigen testing for 12 months.

**Conclusions and Clinical Relevance**—The CR silicone implant containing 7.3 mg of ivermectin was 100% effective in preventing experimental infection with *D immitis* larvae and resulted in negative results for heartworm antigen in a field trial. This product has the potential to alleviate poor owner compliance with monthly prevention regimens. (*Am J Vet Res* 2006;67:1564–1569)

Infection of dogs with the filarial parasite *Dirofilaria immitis* results in canine heartworm disease with high morbidity rates in unprotected dogs in endemic heartworm regions. Canine heartworm disease is treatable; however, prevention is considered the most effective and efficient means of controlling the disease. Ivermectin was the first macrocyclic lactone determined to have activity against *D immitis*.<sup>1</sup> Ivermectin administered every 30 days is effective at preventing canine heartworm infection.<sup>1,2</sup> The orally administered dose was determined on the basis of results of laboratory and field

## ABBREVIATIONS

CR	Covered rod
HPLC	High-performance liquid chromatography
C <sub>max</sub>	Maximum concentration
AUC	Area under the concentration-time curve
L <sub>3</sub>	Third-stage larvae

studies,<sup>3</sup> and the drug is presently recommended for use in dogs at 6  $\mu\text{g}/\text{kg}$  once each month. When used at recommended dose rates, ivermectin is highly active against immature *D immitis* larvae. Since the development of ivermectin, other macrocyclic lactones<sup>4–6</sup> have also been determined to have activity against *D immitis* and are available in orally or topically administered forms, although they still require monthly administration. More recently, a 6-month<sup>7</sup> (marketed in the United States) and a 12-month<sup>8</sup> (marketed in Australia) formulation of moxidectin have been used to provide long-term control of heartworm infection in dogs, which offers greater convenience to pet owners and results in better compliance. However, this product has been associated with concerns regarding its safety and has been withdrawn from the market in the United States pending further FDA consideration. Clark et al<sup>9</sup> also reported on the use of a poly (D, L-lactic-co-glycolic) acid microparticle formulation containing ivermectin to prevent heartworm infections in dogs for up to 6 months.

A sustained-release formulation of ivermectin has been described.<sup>10</sup> The product consists of a cylindrical CR implant with an inner silicone matrix containing ivermectin; the lateral surface is covered with a silicone-only outer layer. Investigation of the release profiles of proteins<sup>11</sup> and ivermectin<sup>10</sup> from CR silicone formulations reveals that the active compound is dissolved and released from the ends of the CR device. Because water does not penetrate the silicone outer cover, it gains entry through the cross-sectional ends and causes dissolution of the active ingredient.<sup>10</sup> The dissolved active ingredient is then free to diffuse and is released through channels formed in the inner core that are created as a result of the dissolution of the active ingredient. The silicone covering therefore controls the release of the active ingredient and further prevents water infiltration, providing zero-order release of the active ingredient. For the hydrophobic drug ivermectin, this results in a sustained zero-order release,<sup>10</sup> but unlike proteins,<sup>11</sup> dissolution of the ivermectin is a rate-limiting factor in release of the drug.<sup>1</sup> Subcutaneous administration of the formulation to mice results in maintenance of a blood concentration

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of ivermectin within 1 order over a period of 3 months.<sup>10</sup> The rate of release of ivermectin is dependent on changing the solubility of ivermectin through use of different additives resulting in zero-order release for 1 year *in vitro*. The purpose of the study reported here was to evaluate use of CR silicone implants containing ivermectin for prevention of infection with *D immitis* in dogs.

## Materials and Methods

Relevant institutional and government animal ethics committees approved all procedures. Dogs were fed and housed according to the National Research Council and National Health and Medical Research Council of Australia Guide for the Care and Use of Laboratory Animals. All dogs had ivermectin concentrations of 0 ng/mL at the start of each study, and the veterinarians and dog attendants ensured that no products containing ivermectin were used at any time.

**Implants**—The implants<sup>a</sup> used in this study were silicone CRs<sup>10,11</sup> with an inner core containing the active ingredient ivermectin<sup>b</sup> (a commercially available formulation consisting of a mixture of not less than 90% ivermectin B1a and not more than 5% ivermectin B1b) and the additives sodium desoxycholate<sup>c</sup> and sucrose<sup>d</sup> in a silicone<sup>e</sup> matrix and an outer wall made of silicone only. The first step in the preparation of the implant is to mix specific amounts of the materials in a preparation mixer to make part A (silicone polymers and catalyst) and part B components (silicone polymers and cross-linker) either with (core) or without (wall) ivermectin. The part A and part B components are mixed to form the core and wall and are coextruded in a clean room by use of an extruder.

When the part A component is mixed with the part B component, the hydride group of the cross-linker reacts with the vinyl group of the silicone polymers in the presence of the platinum catalyst. The result is an ethyl bridge between the polymers with fast curing time, no volatile by-products, and formation of cross-linked silicone-oxide polymer chains. After packaging, the implants are sterilized by use of an electron beam.

**Ivermectin analysis**—Ivermectin (B1a and B1b) in serum or plasma samples stored at  $-20^{\circ}\text{C}$  was analyzed by use of an HPLC method with fluorescence detection.<sup>f</sup> Serum was collected for preclinical studies (studies 1, 2, and 3) and plasma for clinical studies (studies 4, 5, and 6). For plasma samples, lithium heparin was used as an anticoagulant. Ivermectin is very stable when stored frozen, even with repeated freeze thawing. Ivermectin was extracted with acetonitrile. The acetonitrile extract was evaporated to dryness under vacuum, and the residue was dissolved in a hexane-dichloromethane mixture. The organic extract was concentrated by use of a silica column,<sup>g</sup> and ivermectin was eluted with diethylamine ethylacetate. The extracted ivermectin was derivatized with acetic anhydride/1-methylimidazole/dimethyl formamide, and the derivatization mixture was purified by use of a C18 column.<sup>h</sup> The methanol eluate containing ivermectin was concentrated to dryness and made up to volume with the mobile phase prior to quantitation by use of reverse-phase HPLC with fluorescence detection. The limit of quantitation for the assay was 0.1 ng/mL.<sup>f</sup> The method required 1 mL of serum or plasma, and linear calibration graphs ( $r^2 = 0.997$ ) over the range of concentrations studied were used to validate the method. Recovery was  $> 75\%$ , coefficient of variation was 4.10%, and relative residual SDs for accuracy and precision were  $< 8\%$  and  $< 6\%$ , respectively. The amounts of ivermectin B1a and ivermectin B1b were measured, with results expressed as total ivermectin (B1a + B1b).

**Measurement of drug content in CR implants**—After measurement of the weight of the formulation, the formulation was axially cut into 4 sections. Methanol was added at 2 mL/10 mg of formulation. The formulation was then incubated for 3 days at  $37^{\circ}\text{C}$  to elute the ivermectin. The ivermectin concentration in the eluate was measured by use of reverse-phase HPLC to determine drug content in each formulation.<sup>h</sup> Analysis by use of HPLC was performed with a C18 symmetry column<sup>i</sup> (0.46-cm internal diameter  $\times$  5 cm; mean particle size, 3.5 mm), mobile phase A was water, and mobile phase B was methanol. Flow rate was set at 1.5 mL/min. Mobile phase B was increased from 70% to 80% in 4 minutes, and then 80% was retained for 8 minutes. Column temperature was set at  $50^{\circ}\text{C}$ . Detection was performed by measuring absorption at 245 nm.

**Pharmacokinetics**—At various intervals after implantation of the CR, serum of each treated dog was obtained. All dogs received the implant SC through the loose skin between the scapulae. Serum samples were stored frozen ( $-20^{\circ}\text{C}$ ) prior to analysis. Three studies (1, 2, and 3) were undertaken as part of the preclinical development of the CR implant, and  $C_{\text{max}}$  and AUC were determined. The AUC was calculated by use of the linear trapezoidal rule.<sup>12</sup>

**Preclinical study 1**—Dogs were part of a commercial dog breeding operation located in South Eastern Victoria.<sup>j</sup> The percentage of ivermectin and excipients varied with each CR implant. Thirty-six dogs consisting of Beagles and Cocker Spaniels of mixed sex, weighing approximately 20 kg (range, 10 to 27 kg) and between 2 and 7 years of age, were selected. Dogs were housed in fenced yards with shelter. The dogs were fed a mixture of fresh meats consisting of chicken and beef with a high proportion of bone and water *ad libitum*. Six CR implant formulations were tested. Six dogs were allocated to each of the 6 implant formulations (Table 1); serum was prepared from blood collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 16 weeks after implantation; and the amount of ivermectin in the serum was determined by use of HPLC. Dogs were randomly allocated to treatment groups. Dogs in each group were treated with the same length and type of CR implant. Each dog received 10 implants, each measuring 1.6  $\times$  12 mm.

**Preclinical study 2**—For further testing as a potential 12-month product, implant formulations E and F were selected on the basis of results of the first study. For these 2 formulations, implants of 4 lengths each were studied to determine the most appropriate dose for use in dogs. All implants had an external diameter of 1.6 mm. Implants were cut to lengths of 12, 18, 24, or 30 mm. Thirty-two Beagles and Cocker Spaniels, of mixed sex and weighing approximately 20 kg (range, 10 to 30 kg) were selected for 8 groups of 4 dogs each (4 implant lengths and 2 formulations). This study was undertaken in the same location as study 1, and dogs were housed and fed as described for study 1. Dogs received a single implant only. Serum was obtained from blood collected for measurement of ivermectin concentration at 0, 4, 10, 16, 20, 24, 30, 34, 42, 46, 52, and 57 weeks after implantation. Dogs received an ivermectin dose from 4 to 10 mg, depending on the composition of the implant and its length (Table 2).

**Preclinical study 3**—Study 3 was undertaken to determine whether a CR silicone implant (implant E) containing 7.3 mg of ivermectin and measuring 1.6  $\times$  24 mm was sufficient to maintain a putative protective ivermectin concentration<sup>13</sup> against heartworm infection for 52 weeks. This study was undertaken in South Eastern Victoria, per the previous 2 preclinical studies. Dogs in this study were housed and fed as described for study 1. In this study, 14 Beagles (4 females and

10 young adult males; body weight range, 12 to 23 kg) received a single CR silicone implant, and serum samples obtained from blood collected at 0, 1, 2, 4, 8, 10, 12, 21, 33, 37, 52, and 56 weeks were used for measurement of ivermectin concentrations.

**Challenge studies 1 and 2**—Challenge studies 1<sup>k</sup> and 2<sup>l</sup> were randomized controlled trials and had identical formats. Dogs used were hounds, Beagles, and mongrels (body weight range, 18 to 22 kg).<sup>m,n</sup> For each study, 12 dogs (clinical study 1, 12 females; clinical study 2, 6 males and 6 females) received a single 1.6 × 24-mm CR implant containing 7.3 mg of ivermectin<sup>o</sup> on day 0, SC, beneath the loose skin between the scapulae. A further 12 dogs were enrolled in each study as placebo-treated controls that received an implant containing sucrose instead of ivermectin. All dogs were tested for heartworm by use of a commercial heartworm antigen test<sup>p</sup> and modified Knott test. All dogs had negative results for heartworm antigen and microfilaria at the start of the trials. Dogs were monitored for signs of ivermectin toxicosis before implantation and 1, 2, 3, 4, 6, 12, and 24 hours after implantation. After 24 hours, dogs were monitored twice daily for signs of ivermectin toxicosis (10 min/dog). Dogs were observed once daily for the first week for any signs of implant site reactions and again at 30, 90, 180, and 365 days after implantation. On day 365, all dogs received 50 *D immitis* infective L<sub>3</sub> that were cultured and administered according to published methods.<sup>14</sup> The infections were allowed to establish between days 365 and 510. On day 510, all dogs were euthanized and the numbers of adult heartworms in the heart and pulmonary vessels of each dog were counted.<sup>15</sup> Percentage effectiveness of treatment was calculated by comparing the

geometric mean total adult heartworm counts from control and treated dogs according to the following formula:

$$\text{Effectiveness (\%)} = \frac{\text{geometric mean}_{\text{control}} - \text{geometric mean}_{\text{treated}}}{\text{geometric mean}_{\text{control}}}$$

**Field study**—A field study was conducted in Northern Australia<sup>q</sup> with 15 client-owned dogs of mixed breed, age (range, 2 to 9 years), and sex (7 males and 8 females) that weighed between 5.3 and 36.5 kg and were recruited by means of a client database through 2 veterinary practices located in Cairns, Queensland. Dogs were included on the basis of being in good health and having negative results of heartworm antigen tests, body weight from 5 to 40 kg, and a > 12-month history of prophylactic heartworm medication with good compliance history as determined from veterinary practice records. Informed written consent was obtained, and preimplantation blood samples were collected at -4 and 0 weeks for measurement of plasma ivermectin concentrations and heartworm antigen. Heartworm antigen was measured by use of a canine heartworm antigen test kit according to the manufacturer's instructions.<sup>r</sup> Covered-rod implants measured 1.6 × 24 mm and contained 7.3 mg of ivermectin; dogs received implants at a rate of 1 implant/20 kg of body weight (ie, 5- to 20-kg dogs received 1 implant and 21- to 40-kg dogs received 2 implants). Physical examinations were conducted, and blood samples were collected at 0, 1, 18, 26, and 52 weeks after administration of the implant for measurement of plasma concentrations of ivermectin and circulating canine heartworm antigen. A dog with positive results of the heartworm test at ≤ 18 weeks would be excluded from the study because any infection detected at this time would have been caused by pretrial infection.

Table 1—Active drug (ivermectin) and excipient (desoxycholate [DOC] and sucrose [SUC]) composition of 6 CR silicone implants evaluated for heartworm prevention in dogs.

Implant	Ivermectin (%)	DOC (%)	SUC (%)
A	95	0	5
B	90	0	10
C	85	0	15
D	80	0	20
E	90	6.5	3.5
F	80	13	7

Table 2—Dose (mg) of ivermectin used in 2 CR silicone implants (E and F) of various lengths in dogs.

Implant	Length			
	12 mm	18 mm	24 mm	30 mm
E	4.1	6.1	8.2	10.2
F	3.6	5.4	7.2	9.0

Table 3—Mean ± SD pharmacokinetic values for ivermectin obtained from 6 dogs that were each administered 10 CR silicone implants (1.6 × 12 mm) containing ivermectin.

Implant	C <sub>max</sub> (ng/mL)	AUC <sub>0-4</sub> (ng wk/mL)
A	19.2 ± 5.3	61.1 ± 17.3
B	21.0 ± 4.3	62.3 ± 14.1
C	23.0 ± 7.6	63.3 ± 15.3
D	21.5 ± 6.3	63.1 ± 21.7
E	21.0 ± 4.6	61.0 ± 14.1
F	28.4 ± 6.2	81.7 ± 20.9

AUC<sub>0-4</sub> = AUC from weeks 0 to 4.

**Statistical analysis**—Area under the concentration-time curve was calculated from 0 to 4 and 0 to 16 weeks in study 1 and 0 to 57 weeks in study 2 and was determined by use of the linear trapezoidal rule.<sup>s</sup> Comparisons of multiple implants (study 1) were performed via 1-way ANOVA.<sup>t</sup> The AUCs for 4-week and 16-week samples from study 1 were calculated by use of mean serum concentrations for each sampling point. Both 4-week and 16-week determinations were used. Area under the concentration-time curve comparisons were made by use of the 4-week AUC data. Comparisons of AUC and C<sub>max</sub> between implant lengths (study 2) were performed by use of 1-way ANOVA. The AUC and C<sub>max</sub> comparisons between groups that received implants E or F were assessed by use of a Student *t* test for unpaired data. Results with values of *P* < 0.05 were considered significant. Where a significant difference in pharmacokinetic parameters caused by effects of implant size was observed, the data were further analyzed by the use of Fisher protected least squares differences post hoc tests.

## Results

**Preclinical study 1**—Peak mean serum concentrations (19.2 to 28.0 ng/mL) were reached 1 to 2 weeks after implantation. The highest mean concentration (28.0 ng/mL) was observed in the group of dogs given implant F. After reaching peak serum concentration, there was a steady decrease in serum ivermectin concentration for implants A to E, whereas a second peak occurred 4 weeks after implantation for implant F. For dogs that received implants E and F, mean serum ivermectin concentrations at 16 weeks were 8.1 and 6.8 ng/mL, respectively. No significant main effect of implant formulation on AUC (*P* = 0.32) or C<sub>max</sub> (*P* = 0.14) at 4 weeks was detected (Table 3).

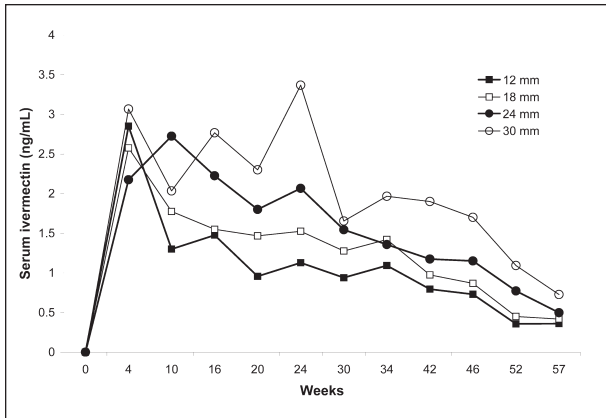


Figure 1—Mean serum ivermectin concentrations in dogs (n = 4/group) that were administered CR silicone implants (implant E [various lengths]) on day 0.

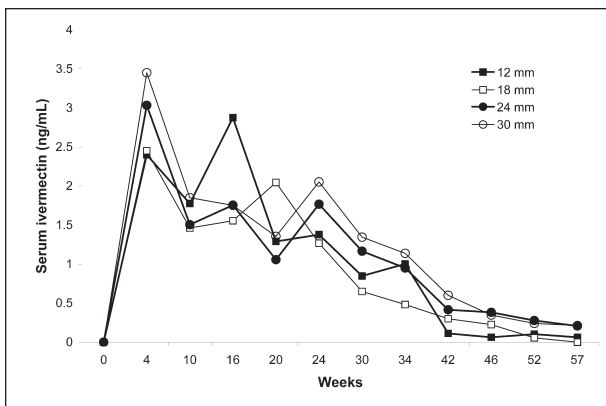


Figure 2—Mean serum ivermectin concentrations in dogs (n = 4/group) that were administered CR silicone implants (implant F [various lengths]) on day 0.

**Preclinical study 2**—Mean serum ivermectin profiles (Figures 1 and 2) obtained from dogs that received E and F implants and pharmacokinetic values were determined (Table 4). A significant ( $P = 0.03$ ) main effect of implant length on AUC but not Cmax ( $P = 0.58$ ) was detected. Fisher protected least squares difference post hoc tests revealed a significantly greater difference in AUC between implants of 30 mm and 12 mm ( $P = 0.005$ ) and between implants of 30 mm and 18 mm ( $P = 0.02$ ). Mean AUC for weeks 0 to 57 ranged from 60.5 ng•wk/mL to 115.2 ng•wk/mL.

Mean Cmax for implants of different lengths ranged from 2.6 to 3.9 ng/mL. No significant differences were detected in serum ivermectin concentrations between implants E and F when administered at the same size ( $P = 0.93, 0.35, 0.10,$  and  $0.14,$  for 12-, 18-, 24-, and 30-mm lengths, respectively).

Although there were no significant differences between formulations E and F with respect to pharmacokinetic variables, there were differences in maintenance of serum ivermectin concentration  $\geq 0.2$  ng/mL for all dogs in each group. All dogs given E implants, at either 24 mm or 30 mm, had serum ivermectin concentrations  $\geq 0.2$  ng/mL at 52 weeks, with mean serum ivermectin concentrations of 0.77 and 1.09 ng/mL respectively. Mean ivermectin concentration was  $> 0.2$

Table 4—Mean  $\pm$  SD Cmax and AUC (0 to 57 weeks) for dogs (n = 4/group; groups E and F) administered a single CR silicone implant of different lengths containing ivermectin.

Variable	Length			
	12 mm	18 mm	24 mm	30 mm
Cmax (ng/mL)				
E	2.85 $\pm$ 1.5	2.58 $\pm$ 1.1	2.90 $\pm$ 0.5	3.90 $\pm$ 1.7
F	3.50 $\pm$ 1.5	2.45 $\pm$ 1.7	2.58 $\pm$ 1.1	3.53 $\pm$ 0.7
AUC (ng wk/mL)				
E	60.5 $\pm$ 6.7 <sup>a</sup>	71.3 $\pm$ 20.9 <sup>a</sup>	87.5 $\pm$ 14.7 <sup>ab</sup>	115.2 $\pm$ 34.6 <sup>b</sup>
F	61.0 $\pm$ 9.7	48.9 $\pm$ 39.3	61.1 $\pm$ 22.5	73.5 $\pm$ 28.2

<sup>a,b</sup>Within a row, values with different superscript letters are significantly ( $P < 0.05$ ) different.

Table 5—Mean number of male and female heartworms recovered on day 510 from control dogs (n = 12/group) and dogs (12/group) treated with a CR silicone implant containing 7.3 mg of ivermectin on day 0 and challenged by inoculation with 50 L<sub>3</sub> *Dirofilaria immitis* on day 365 in 2 studies.

Challenge study No.	Male	Female	Total
1			
Control	12.2	16.4	28.6
Treated	0	0	0
2			
Control	14.9	16	30.9
Treated	0	0	0
1 and 2 combined			
Control	13.5	16.2	29.8
Treated	0	0	0

ng/mL for all dogs given E implants (any length) and F implants at 24 mm and 30 mm. Ivermectin was not detectable in approximately half the dogs receiving implant F at 24 or 30 mm and in dogs receiving implant E at 12 or 18 mm.

**Preclinical study 3**—Serum ivermectin concentrations of 14 dogs from 0 to 56 weeks after implantation with a single CR implant were measured. Mean serum ivermectin concentrations at 52 and 56 weeks were 0.5 and 0.7 ng/mL, respectively; the range at 52 weeks was 0.23 to 1.1 ng/mL and at 56 weeks was 0.44 to 1.8 ng/mL.

**Clinical studies 1 and 2**—A mean of 29.8 adult heartworms/dog were recovered from the 24 dogs in the 2 control groups (Table 5). No adult heartworms were recovered from the 24 treated dogs. Mean plasma ivermectin concentration of treated dogs at challenge (ie, 365 days after implantation) was 0.4 ng/mL (range, 0.15 to 0.64 ng/mL). A single silicone implant containing 7.3 mg of ivermectin was 100% effective in preventing infection with 50 infective L<sub>3</sub> *D immitis* inoculated 12 months after treatment.

**Field study**—Thirteen of the 15 dogs enrolled in the study completed the full 12 months of the study. One dog was hit by a car and killed and was consequently lost from the study. A second dog had a positive heartworm antigen test result at 18 weeks and was excluded from the study; a subsequent canine heart-

worm antigen test from a repeat plasma sample yielded negative results. Of the 13 dogs that completed the field study, all had plasma concentrations of ivermectin  $> 0.2$  ng/mL 52 weeks after implantation. Mean plasma ivermectin concentrations at 1, 18, and 26 weeks after implantation were  $5.6 \pm 2.93$  ng/mL,  $1.07 \pm 0.64$  ng/mL, and  $1.04 \pm 0.39$  ng/mL, respectively. Mean plasma ivermectin concentration at 52 weeks was  $0.89 \pm 0.61$  ng/mL (range, 0.37 to 2.8 ng/mL). None of the 13 dogs had detectable circulating heartworm antigen at 78 weeks after implantation.

## Discussion

Members of the macrocyclic lactone family are highly effective in preventing heartworm infection when used consistently, irrespective of their mode of application.<sup>16</sup> The products are also extremely safe because they are used at very low concentrations. Nevertheless, their effectiveness is often compromised by lack of owner compliance, which exposes dogs to infection at critical times of the year.<sup>17</sup> Additionally, shifting weather patterns may alter the distribution and concentration of vectors responsible for transmission of *D immitis*, making seasonal administration of these compounds a less reliable means of preventing infection. Year-round administration is thought to avoid the difficulties associated with correct timing of initiation and cessation of preventative administration. Development of an injectable form of moxidectin that provides protection for 6 months was intended to remove lack of owner compliance from prevention programs; however, not all regions have a transmission period as short as 6 months. Development of a product providing 12 months of protection that is both safe to use and efficacious would be of considerable benefit to dog owners and veterinarians.

*Dirofilaria immitis*, especially the fourth-stage larvae, are susceptible to ivermectin. On the basis of results of laboratory and field studies, it has been determined that ivermectin administered at  $6 \mu\text{g}/\text{kg}$  PO, once per month, provides protection against canine heartworm disease.<sup>3</sup> Even when administered every 2 to 3 months, ivermectin remains highly efficacious in preventing canine heartworm infection, and doses of 1 to  $2 \mu\text{g}/\text{kg}$  have good efficacy in field studies.<sup>3</sup> In a study conducted by Daurio et al,<sup>13</sup> ivermectin administered PO at  $6 \mu\text{g}/\text{kg}$  reached a peak plasma concentration of approximately 3.0 ng/mL in 2 to 4 hours and this concentration decayed exponentially after this. Within 72 hours of its administration, plasma concentration was approximately 0.5 ng/mL and by 14 days had decreased to  $< 0.2$  ng/mL. On the basis of these considerations, it was anticipated that an implant delivering a serum concentration of  $\geq 0.2$  ng/mL would provide 100% protection against canine heartworm infection. It is not known whether lower concentrations of ivermectin would be required when administered in a continuous fashion (as with CR implants), as opposed to the pulsatile release from monthly oral administration.

Administration of ivermectin results in a proportional relationship between the dose administered and the serum concentration attained, when the dose is

within the standard therapeutic range. The serum concentration of ivermectin measured in dogs given 10 implants (preclinical study 1) was 10 times the serum concentration achieved after administration of commercial products that contain ivermectin for heartworm control in dogs.<sup>13</sup> The dose of ivermectin delivered from each CR implant was greater than the putative therapeutic value, suggesting that each CR implant resulted in a peak serum concentration of 2.5 to 3.0 ng/mL, similar to that observed after administration of ivermectin PO at  $6 \mu\text{g}/\text{kg}$ .<sup>13</sup> This was supported by the finding in the second preclinical study that dogs given a single CR implant ( $1.6 \times 12$  mm) identical in composition to implants used in the first study had a  $C_{\text{max}}$  from 2.9 to 3.0 ng/mL.

Changes in the dissolution additives can be used to increase the serum concentration of ivermectin. However, care must be taken to ensure that the rate of release, although resulting in therapeutic concentrations of ivermectin, is not too rapid. Comparison of the single CR implants E and F in preclinical study 2 revealed that there was little difference in the  $C_{\text{max}}$  irrespective of the amount of sodium desoxycholate in the 2 formulations. Administration of both formulations resulted in serum ivermectin concentrations greater than the theoretical minimal therapeutic concentration. There were, however, significant differences between the 2 formulations in the duration that the therapeutic concentrations were maintained. In preclinical study 2, differences in the length (ie, 24 or 30 mm vs 12 or 18 mm) of implant were associated with differences in sustained duration of therapeutic concentrations of ivermectin without any differences in  $C_{\text{max}}$ . This observation was similar to results of Maeda et al,<sup>10</sup> who reported that for hydrophobic ivermectin, the ivermectin is only released from the ends of the CR implant because the outer silicone layer provides an impermeable barrier to drug dissolution and release. For CR implants that contain ivermectin, the cross-sectional area restricts release. Implants with the same formulation and same cross-sectional area but of different lengths yield the same rate of release, despite the greater gross amount of active drug in longer implants, because the percentage of ivermectin remains unaltered, along with the rate of dissolution. The extended release profile of longer implants is attributable to the greater amount of ivermectin contained in the implant and the increased time required for water to penetrate the entire length of the implant and dissolve the drug.

Results of clinical studies 1 and 2 indicated that the implants were highly efficacious in preventing infection with *D immitis* in dogs challenged on day 365 after administration of the implants with high numbers of larvae, which are not likely to be encountered via natural challenge. Plasma concentrations of ivermectin at the time of challenge (ie, 365 days after administration) ranged from 0.15 to 0.64 ng/mL. Hence, a plasma concentration of approximately 0.2 ng/mL can be considered to provide 100% protection against heartworm infection in dogs. The lowest protective concentration of ivermectin cannot be concluded from these studies. Plasma concentrations  $< 0.2$  ng/mL may be equally

effective, and in support of this, a single treated dog with a plasma ivermectin concentration of 0.15 ng/mL was found to be free of infection at necropsy.

Effectiveness of the implants was evaluated in a field study undertaken in Northern Australia, an area with historically high prevalence of canine heartworm infection. Heartworm infection was not detected in any of the dogs. Plasma concentrations of ivermectin at 52 weeks were > 0.2ng/mL.

The higher plasma concentrations of ivermectin in dogs in the field study were most likely attributable to most dogs receiving 2 implants at day 0 because of body weights > 20 kg. In this situation, the concentrations derived from each implant were additive. In 1 dog in the field study, a 52-week plasma ivermectin concentration of 2.8 ng/mL was observed, reflecting the much smaller size of this dog (5 kg).

None of the dogs (n = 120) treated with CR implants had any adverse systemic reactions, even when 10 implants were administered. No swellings were detected at the site of implantation. Veterinarians and dog owners participating in the field trial did not report any concerns or signs of discomfort in their dogs.

Some breeds of dogs, in particular Collies,<sup>18</sup> have increased susceptibility to the toxic effects of ivermectin, which is caused by a defect in the multi-drug-resistance gene (*MDR1*) that encodes for P-glycoprotein, an integral component of the blood-brain barrier,<sup>19</sup> resulting in high concentrations of ivermectin in the brain. Toxicosis is observed when Collies are given ivermectin at concentrations 15 times the therapeutic dose. The CR implants used in the study reported here resulted in a peak plasma concentration from 3 to 7 ng/mL; this concentration of ivermectin is not likely to result in toxicosis in Collies.

- a. Smart Drug Systems Inc, Pawcatuck, Conn.
- b. Ivermectin, Zhejiang Hisun Pharmaceuticals Ltd, Taizhou City, People's Republic of China.
- c. Sodium desoxycholate, International Specialty Chemicals, Palmerston North, New Zealand.
- d. Sucrose, Mallinckrodt Baker, Phillipsburg, NJ.
- e. Silicone polymers, cross-linkers and platinum catalyst, Nusil Technologies, Carpinteria, Calif.
- f. NM-6, Australian Government Analytical Laboratories, Cottesloe, Western Australia, Australia.
- g. Sepak, Merck & Co, Rahway, NJ.
- h. Laboratory analysis performed by PPD Development, Middleton, Wis.
- i. Novapak C18 Column, Waters, Milford, Mass.
- j. ACA Breeders, Victoria, Australia.
- k. Professional Laboratory and Research Services Inc, Corapeake, NC.
- l. TRS Laboratories, Athens, Ga.
- m. Covance Research Products Inc, Kalamazoo, Mich.
- n. Alder Ridge Farms Inc, Lakewood, Pa.
- o. Lot No. CI-08-09-03, Smart Drug Systems Inc, Pawcatuck, Conn.
- p. DiroCHEK, Synbiotics Corp, Animal Health Division, San Diego, Calif.
- q. Cairns, Queensland, Australia.
- r. AGEN Biomedical Pty Ltd, Brisbane, Australia.
- s. Excel 97, Microsoft Corp, Redmond, Wash.

t. Statview for Macintosh, version 5.0.1, SAS Institute Inc, Cary, NC.

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