Activity of an injectable, sustained-release formulation of moxidectin administered prophylactically to mixed-breed dogs to prevent infection with *Dirofilaria immitis*

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Objective—To test the ability of a single injection of a sustained-release formulation of moxidectin (moxidectin SR) to protect dogs against heartworm infection for 180 days after inoculation with infective third-stage larvae (L3) of *Dirofilaria immitis*.

Animals—32 adult mixed-breed dogs.

Procedure—Dogs were allocated to 4 groups on the basis of weight and sex. Dogs were injected SC with saline (0.9% NaCl) solution or moxidectin SR at the rate of 0.06, 0.17, or 0.5 mg/kg of body weight (day 0). Each dog was inoculated SC with 50 *D immitis* L3 180 days later. On days 330 and 331, dogs were euthanatized. The heart, lungs, and thoracic cavity were examined, and number and sex of heartworms were determined.

Results—A mean of 35.9 heartworms was recovered from untreated control dogs. Fourteen worms were recovered from 1 of 8 dogs given moxidectin SR at the lowest dosage, and none of the dogs in the 2 highest moxidectin treatment groups were infected. Small barely palpable granulomas were detected at injection sites of moxidectin-treated dogs. Frequency and size of granulomas were positively correlated with dose of moxidectin administered.

Conclusions and Clinical Relevance—A single dose of moxidectin SR at a dosage as low as 0.17 mg/kg can safely and reliably confer complete protection against infection after challenge-exposure with *D immitis* L3, and protection lasts for at least 180 days. This mode of prophylactic treatment against infection with heartworms effectively eliminates failure of prophylaxis that results from erratic administration of medications designed for monthly administration. (*Am J Vet Res* 2001;62:1721–1726)

Dirofilaria immitis can cause debilitating, potentially fatal cardiopulmonary disease in dogs and cats. Infection with *D immitis* results when mature **third-stage larvae (L3)** gain entry to a host during feeding by vector mosquitoes. Dogs and cats are at risk when a reservoir of microfilaremic hosts exists, there is a sufficient population of vector-competent mosquitoes, and there is a sufficient environmental temperature to allow maturation of L3 in these vectors. Because of the mobility of domestic dogs, which are the primary reservoir for infection, and the ubiquity of potential vector mosquitoes, the geographic range of *D immitis* in North America is extensive. In the preceding 4 decades, it has expanded from the southeastern and gulf-coast states to encompass the Mississippi River basin; states in the mid-Atlantic, northeastern, and southwestern United States; foci on the Pacific Coast; and the southern tier of Canadian provinces.

Since the early 1960s, chemoprophylaxis for infection with heartworms has evolved in stages to reach a situation in which less frequent administration of drugs is required; this is achieved by the use of various dosing formulations created to facilitate administration. The first effective chemoprophylactic developed to prevent *D immitis* infection of dogs[1-3] was **diethylcarbamazine citrate (DEC)**. Successful prophylaxis with DEC depends on consistent daily administration begun prior to infection and maintained for 60 days following the period of transmission. Omission of even a few doses can result in failure of protection.

Macrolide endectocides constitute a second generation of chemoprophylactics against infection with heartworms. These compounds are highly active against the **tissue-migrating stage (L4)** of *D immitis* and are virtually 100% effective up to the sixth week of infection.[4-6] The strategy underlying their use as chemoprophylactic agents does not involve maintaining lethal amounts of active compound in tissues or plasma, as is the situation for DEC. Rather, administration is aimed at periodically destroying all developing larvae early during infection prior to their entrance into the circulation and exertion of a pathogenic effect. Ivermectin, the first compound of this class to be licensed as a chemoprophylactic against infection with heartworms, was initially marketed in 1987 as an oral formulation designed to deliver an effective dose when administered at monthly intervals during the transmission season.[4,7,8] Other members of this class, including milbemycin oxime,[5,9] moxidectin,[6,10,11] and selamectin,[12] also are effective as oral or topical formulations administered on a monthly basis. Because of their broad spectrum of activity against various stages of heartworm larvae, macrolide endectocides generally offer a safety margin that enables an owner to miss administration of

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1 dose without a lapse in protection, provided subsequent doses are given on schedule. Nevertheless, successful chemoprophylaxis with these compounds depends ultimately on a certain degree of client compliance with the prescribed monthly dosing regimen.

One method of obviating an extended program of repetitive administration has been the use of sustained-release devices or formulations. A timed-release ivermectin bolus reportedly controls ectoparasites such as *Psoroptes ovis*[13] and gastrointestinal helminths such as *Haemonchus* spp, *Trichostrongylus* spp, and *Cooperia* spp in ruminants.[14] The effectiveness of an injectable microencapsulated formulation of ivermectin in the control of certain ticks (*Amblyomma americanum*) and horn flies (*Hematobia irritans*) also has been reported.[15] Those same investigators suggested that such a slow-release microsphere formulation of ivermectin could be useful in chemoprophylaxis against infection with heartworms. In the study reported here, we evaluated such a sustained-release formulation of moxidectin to determine whether it can effectively protect dogs that were challenge-inoculated with *D immitis* L3 180 days after administration.

Materials and Methods

Animals—Thirty-two (16 male and 16 female) purpose-bred mixed-breed dogs were obtained from a commercial vendor.[a] Dogs were at least 8 months old and had been reared under parasite-free conditions. They did not have previous exposure to mosquitoes and had not been treated with any macrolide endectocide. From the time of their arrival at our facility until the end of the study, dogs were housed in runs (2 dogs/run). Male and female dogs were housed in separate rooms. The study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

All dogs were quarantined and conditioned during a 14-day period prior to treatment. During this period, blood samples were obtained for a CBC, and fecal samples were collected from each pair of dogs. Two days prior to treatment, blood samples were obtained from each dog and tested for evidence of adult heartworm antigen, using a commercially available test kit,[b] and for evidence of circulating *D immitis* microfilariae, using the modified Knott test.

Two days prior to treatment, male dogs were weighed and ranked on the basis of body weight. Males were then allocated into 4 weight classes, and 1 member of each class was randomly assigned to each of 4 treatment groups. This process then was repeated with the female dogs. The final result was 4 treatment groups containing 8 dogs that comprised equal numbers of males and females from each of 4 weight classes. Following allocation to treatment groups, male and female dogs continued to occupy their respective rooms in the facility. However, within those rooms, the 4 members of each treatment group were housed in pairs in 2 adjacent runs.

Procedures—Moxidectin SR, a proprietary sustained-release formulation of moxidectin microspheres (10%; wt:wt),[c] was used in the study. Analysis of the product indicated that the actual concentration of moxidectin was 10.7% (wt:wt), which was within specification limits for the product. The vehicle for the microspheres was a proprietary moxidectin sustained-release vehicle.[d] Bacteriostatic saline (0.9% NaCl) solution was used to treat dogs in the negative-control group.

Proposed injection sites were clipped 2 days prior to treatment; these sites were on the left side of the neck of each dog immediately cranial to the scapula. On day 0, 10% moxidectin microspheres were constituted in vehicle in accordance with a predetermined protocol (**Appendix**). Constitution in this manner resulted in suspensions containing approximately 12, 33, or 100 mg of microspheres/ml. Injection of these suspensions at a volume of 0.05 ml/kg of body weight resulted in doses of approximately 0.06, 0.17, or 0.5 mg of moxidectin/kg, respectively. Each of the 4 groups was randomly assigned to receive 1 of the treatments. Microsphere suspensions or equal volumes of saline solution were injected SC into the clipped injection sites (day 0). Personnel who assigned treatments to the dog groups and who performed the injections did not participate in subsequent evaluations. Thus, the investigators who performed evaluations were not aware of the treatment status of the dogs.

Challenge inoculation with *D immitis* L3—The source of *D immitis* for challenge inoculations was a naturally infected dog that did not have clinical signs of disease. The dog was acquired from a commercial vendor.[e] Records indicated that the dog had originally been infected in the locality of this vendor.

Dirofilaria immitis L3 were grown in laboratory-reared *Aedes aegypti* of the Liverpool-selected strain, which is highly susceptible to infection.[16] Mosquitoes were reared by use of standard techniques,[17] and females were infected via feeding on an artificial membrane, using microfilaremic blood obtained from the naturally infected source dog that had been diluted to a concentration of 6×10^3 parasites/ml by the addition of noninfected canine blood. Infected mosquitoes were maintained in darkness at 80% relative humidity and were fed 10% (wt:vol) aqueous sucrose. Cohorts of infected mosquitoes were incubated for 7 days at 30 C, 13 days at 27 C, or 20 days at 25 C. Infection of these mosquitoes was timed so that all harbored L3 on day 180.

On the day of inoculation (day 180), infected mosquitoes were narcotized with CO_2, and the surface of each mosquito was decontaminated by immersion for 3 minutes in 1% benzalkonium chloride in 0.01*M* PBS solution. Mosquitoes then were washed twice in sterile PBS solution and transferred to dishes containing a sterile medium containing equal parts of 2 standard mammalian tissue culture media (ie, **NCTC-135**[f] **and Iscoves modified Dulbecco medium [NI]**[g]).[18] Mosquitoes were incubated without dissection for 10 minutes at 37 C to promote egress of L3 from the mouthparts. Emergent L3 were collected into sterile flame-constricted Pasteur pipettes, washed once in sterile NI medium, and transferred in groups of 50 to the barrels of sterile 1-ml syringes fitted with 18-gauge needles. The L3 were inoculated SC into the flank of each dog. Then, each syringe was refilled with sterile NI, which was injected into each dog. Used syringes were labeled with the identification of the dog; these syringes were rinsed with NI medium, and any residual parasites were counted and discarded. In a few instances, L3 were retained in syringes; a corresponding number of fresh L3 was injected into the appropriate dog, using another syringe.

Blood collections for moxidectin assay and heartworm testing—On days –2, 8, 14, 28, 55, 84, 112, 140, 181, 204, 231, 268, 300, and 329, approximately 10 ml of blood was collected by venipuncture, and a serum sample from each dog was frozen at –20 C. Samples were shipped overnight on dry ice to a laboratory[h] for analysis of moxidectin concentrations. Moxidectin concentrations were determined, using an assay method[i] that has a **limit of quantitation (LOQ)** of 0.5 ng/ml. The method used has been described by Alvinerie et al.[19] The method requires 1 ml of serum and linear calibration graphs (r = 0.997) over the range of concentrations studied (ie, 0.1 to 10 ng/ml). Recovery data reported elsewhere[19] indi-

cate that at low concentrations, recovery is > 75%, whereas at concentrations > 0.2 ng/ml, recovery is approximately 100% with a relative SD of approximately 6%. In addition, blood and serum samples were obtained on days 178 and 329 and examined for *D immitis* microfilariae and adult heartworm antigen, using methods described for the samples obtained on day –2.

Analysis of moxidectin concentrations—Nonlinear least-squares approximations were used to fit the data to mathematical models of the following equation:

$$C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + C_3 e^{-\lambda_3 t}$$

where C is the blood concentration at time *t*.[20]

The calculated coefficients, C_1, C_2, and C_3, are subject to the open model assumption that $C_3 = C_1 + C_2$. The exponent variables, λ_1, λ_2, and λ_3, are first-order rate constants. The first term corresponds to the terminal phase of the blood concentration curve. The third term corresponds to the initial increase in concentration following dosing. The second and third terms together are empirical fits to the data and are interpreted only to approximate a complex relationship between microsphere release, absorption into the blood, and distribution and equilibrium among body compartments such as blood, rapidly perfused tissues, and fatty tissue. That is, the terms that mathematically describe the initial and middle phases are interpreted to be composite terms describing a potential multitude of physiologic processes. One-to-one physiologic analogues are not assigned to these terms. For example, we do not interpret the rapid increase in concentration to absorption alone, because release from micro spheres soon after injection is theoretically not a first-order reaction and potentially relatively rapid.

Antemortem observations—All dogs were observed at hourly intervals for the first 6 hours following treatment with moxidectin SR for signs of adverse reactions. Thereafter, dogs were observed twice daily on days 1 to 7 of the study.

Each injection site was examined for evidence of swelling at 5 hours after administration of moxidectin SR, then daily for 2 weeks, and thereafter at 2- to 3-week intervals for the duration of the study. Swelling was serially evaluated by a scoring system that used visual appearance, palpable characteristics, consistency, size, and any increase in swelling since the last observation.

Postmortem examination—Dogs were euthanatized with an overdose of pentobarbital on days 330 (males) and 331 (females). A skin sample containing the injection site was excised from each carcass and preserved in buffered 10% formalin. To standardize the size of these skin samples, the dog with the largest lesion in each group was processed first. Tissue samples collected from all dogs necropsied that day were of sufficient size to include the largest lesion plus 0.5 cm of skin surrounding the lesion.

During necropsy, a clamp was placed on the vena cava, and the heart, lungs, and associated blood vessels were removed and transferred to a separate tray. The peritoneal and thoracic cavities also were examined closely for heartworms. Abnormalities in the heart or lungs were recorded. These organs were dissected as described by McCall et al[8] and examined for adult heartworms, which were removed and placed in dishes containing 0.01*M* PBS solution. Heartworms were sorted on the basis of sex, counted, and classified as alive, stunted, or dead. A few worm fragments were recovered, but only fragments that contained a head were counted as worms. All recovered worms were preserved in buffered 10% formalin.

Histologic examination—Skin samples obtained from injection sites were allowed to remain in fixative for a minimum of 24 hours. Then, 2 representative segments were excised from each sample. These were dehydrated in a graded series of ethanol dilutions, cleared, and embedded in paraffin. Sections (5-µm thick) were cut from each block, placed on slides, stained with H&E, and covered with a coverslip. Two representative sections from each sample were examined for evidence of lesions. Lesions were described in a written narrative, characterized, and graded on the basis of severity, using a 3-point scale (1 = mild; 2 = moderate; 3 = severe).

Results

Parasitologic findings—Adult *D immitis* recovered from dogs in the 4 treatment groups during necropsy were recorded (**Table 1**). A mean of 35.9 heartworms was recovered from the 8 dogs in the control group. This represents a recovery rate of 70% of the L3 inoculated. Mean recovery of 1.75 adult heartworms from the group of dogs that received the lowest dose of moxidectin (0.06 mg/kg) was attributable to a single infected dog from which 14 live worms were recovered. Heartworms were not recovered from any other dog in that treatment group, nor were they recovered from dogs receiving higher dosages of moxidectin (0.17 or 0.5 mg/kg).

Table 1—Mean No. of heartworms recovered from dogs challenge inoculated with *Dirofilaria immitis* infective third-stage larvae (L3) 180 days after treatment with a sustained-release formulation of moxidectin (moxidectin SR) or saline (0.9% NaCl) solution (control dogs)*

Moxidectin (mg/kg of body weight)	No. of infected dogs	Mean No. of heartworms recovered per dog			
		Males†	Females†	Sex unknown	Total
0	8	1.0	19.0	0.9	35.9 (28–39)
0.06	1	0.5	0.9	0.4	1.8 (0–14)
0.17	0	0	0	0	0
0.50	0	0	0	0	0

Values in parentheses represent the range.
*There were 8 dogs in each group. Dogs were inoculated with 50 *D immitis* L3. †No. of whole heartworms. ‡No. of heartworm fragments that contained a head.

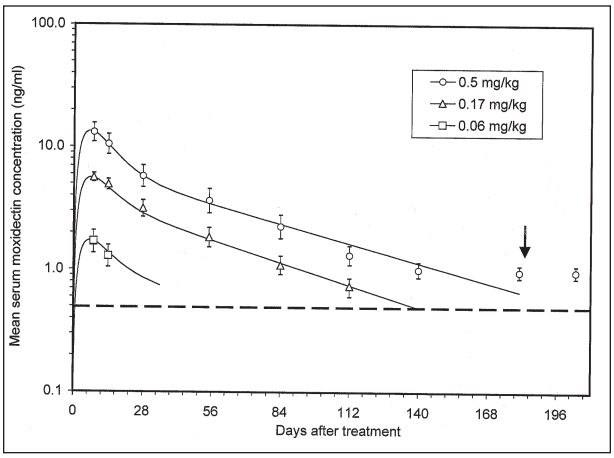

Figure 1—Mean ± SEM moxidectin concentrations in serum samples obtained from dogs 8 to 329 days after treatment with various doses of a sustained-release formulation of moxidectin. Means of the groups were estimated when at least 6 of 8 dogs in a group at the time of sample collection had moxidectin concentrations that were greater than the level of quantitation (0.5 ng/ml; dashed line). Day of challenge inoculation with infective third-stage larvae of *Dirofilaria immitis* is indicated (arrow).

Serum moxidectin concentrations—Serum concentrations of moxidectin peaked on day 8 (**Fig 1**). Values were proportional to the dosage of moxidectin SR administered. Thereafter, moxidectin concentrations declined over the course of the study. When serum moxidectin concentrations of 1 or, infrequently, 2 of the 8 dogs in a treatment group decreased to less than the LOQ, estimates of the mean for the group could not be made and were not plotted. This was evident on days 55 and 140 for the groups that received 0.06 and 0.17 mg/kg, respectively.

Coefficients and exponents for equations describing the curve for each of the 3 treatment groups were calculated (**Table 2**). The λ_1 value was approximately –0.014/d, which corresponds to a relatively long terminal half-life of approximately 50 days. The λ_1 value was similar for the 2 higher doses and was evident graphically, because the 2 curves were essentially parallel. On the basis of the parallel nature of the curves for the 2 higher doses and other anecdotal data for concentrations in dogs treated with these microspheres, the terminal phase for the lowest dose also was essentially parallel to the higher doses.

Table 2—Values of coefficients (C_1, C_2, and C_3) and first-order rate constants describing kinetics of serum moxidectin concentrations in dogs treated with various doses of moxidectin SR

	Dosage of moxidectin SR (mg/kg)		
Variable	**0.5**	**0.17**	**0.06**
Terminal phase			
C_1	7	4	1.22
λ_1	–0.013	–0.015	–0.015
Middle phase			
C_2	36	13.5	5.5
λ_2	–0.13	–0.14	–0.16
Initial phase			
C_3	–43	–17.5	–6.72
λ_3	–0.25	–0.25	–0.25

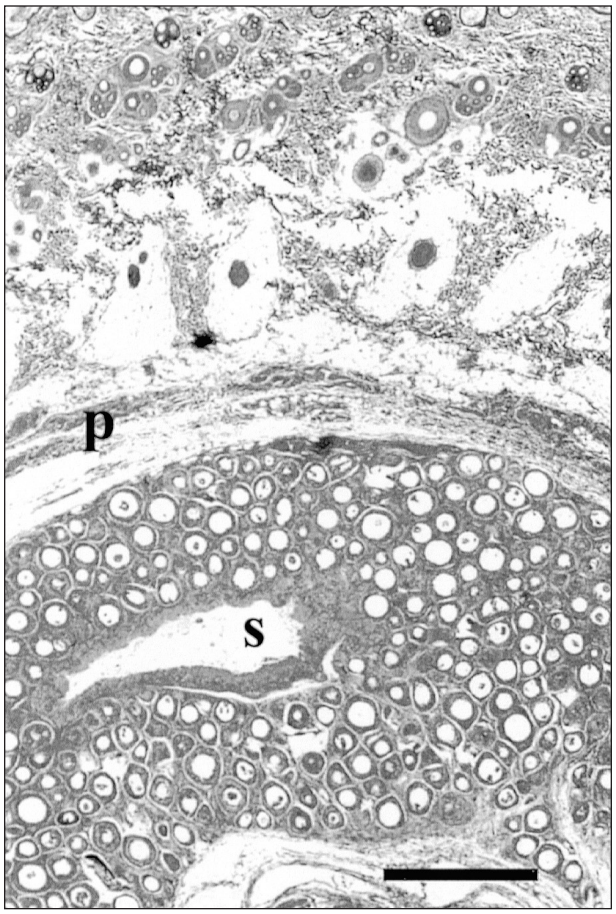

Figure 2—Photomicrograph of a section of skin from a dog revealing a granulomatous lesion consistent with a foreign-body reaction at the site of injection 330 days after treatment with a sustained-release formulation of moxidectin. A focus of granulomatous inflammation centered around a large partially empty central space (s) is located in the subcutis beneath the panniculus muscle (p). This focus contains numerous smaller-sized clear circles representing deposits of fatty material, consistent in size with moxidectin SR microspheres, that are rimmed by compressed epithelioid macrophages and multinucleated giant cells. H&E stain, bar = 1 mm.

Antemortem observations—Adverse reactions attributable to treatment with moxidectin SR were not observed in any dog during the course of the study. All dogs injected with moxidectin SR or saline solution had small swellings or focal hemorrhages at the injection site beginning on day 1 and persisting in some cases until day 13. After that time, a miniscule lump was detected on day 21 in 1 control dog and on day 140 in some dogs that had received moxidectin SR (0.17 mg/kg). Only dogs that had received the high dose of moxidectin SR (0.5 mg/kg) had swelling that was initially visible beneath the freshly clipped skin and palpable from days 3 or 4 until the final observations on day 329. These lumps consisted of firm, slender, and oblong tracks or chains of tiny nodules (mean maximum length, 1.9 cm) in the subcutaneous tissues that progressively shrank to an approximate length of 0.5 cm, which made it difficult to locate them.

During the quarantine period, a pooled fecal sample from each pair of dogs housed in the same run was examined for evidence of gastrointestinal parasites. *Giardia lamblia* cysts were found in 8 of 16 pooled fecal samples. Because of the high (minimum, 25%) frequency of infection with *Giardia* spp and the confined housing conditions, all dogs were treated with a course of metronidazole (30 mg/kg, PO, q 24 h for 5 days) after which results were negative for analyses of all subsequent fecal samples. Because of an accidental minor laceration, 1 dog in the high-dose moxidectin SR treatment group was administered amoxicillin clavulanate (375 mg/kg, PO, q 24 h for 10 days).

Postmortem examination—Gross lesions in the

Table 3—Frequency and severity of granulomatous lesions associated with a subcutaneous injection of moxidectin SR in groups of dogs (n = 8 dogs/group)

Moxidectin (mg/kg of body weight)	No. of dogs with lesions	Mean lesion score*	No. of dogs with other findings†
0	0	0	2
0.06	3	0.5	2
0.17	4	1.0	4
0.50	6	2.1	2

*Scale of 0 to 3 for severity (1 = mild; 2 = moderate; and 3 = severe). †Other findings included mild inflammation of the superficial dermis, mild atrophy of the panniculus muscle, and a focal deep granuloma encircling a naked hair.

lungs, consistent with *D immitis* infection, were observed during necropsy in 5 of 8 control dogs, all of which were infected, and in 1 infected dog in the lowest moxidectin SR treatment group. Examination of tissues from the injection sites revealed granulomatous lesions consistent with a foreign substance in all 3 groups treated with moxidectin SR (**Fig 2**). The frequency of these lesions and their severity, expressed as mean lesion scores, were related to the dose of moxidectin SR administered (**Table 3**). In contrast, frequencies of other findings were not related to the dose of moxidectin SR.

Discussion

Analysis of the results of the study reported here indicated that at dose rates of 0.17 or 0.5 mg/kg, a single SC injection of moxidectin SR conferred 100% protection against challenge inoculation with *D immitis* L3 180 days after prophylactic administration. Seven of 8 dogs treated at the lowest dose of 0.06 mg/kg also were completely protected; the 1 infected dog in this treatment group had 14 heartworms. That value represents 39% of the mean recovery of 35.9 worms from dogs in the control group.

The moxidectin concentration in the serum of the dog in which failure of protection was observed was at the LOQ 8 days after treatment and was less than the LOQ thereafter. By contrast, detectable concentrations of drug were reported in the sera of the other dogs in this group until at least day 14 and, for most of those dogs, as long as 55 days. This fact, along with the nonuniform distribution of parasites among dogs in the group receiving 0.06 mg/kg, suggests that the failure of protection observed in this group may have been the result of individual variation in pharmacokinetics or titration of the active ingredient to the limit of efficacy. Injection mishaps did not occur in the study.

None of the dogs treated with moxidectin SR had adverse systemic reactions. Swelling at the injection sites was palpable in some of the dogs receiving the 2 lowest dosages of moxidectin SR during the first 13 days after administration, but it was barely detectable. On the basis of serial observations of injection sites in the group receiving the highest dosage of moxidectin SR, the subcutaneous swellings reached maximum size in terms of being visible and palpable at 2 weeks after administration and gradually shrank thereafter. Had the hair over the injection sites not been clipped, it would not have been possible to recognize the subtle visual evidence of swelling, and without knowing precisely where to search, it is unlikely that even the largest lumps would have been detected during a generalized palpation. The local foreign body reaction in the moxidectin SR-injected dogs appeared to be dependent on the volume of microspheres at the site rather than the concentration of active ingredient per unit of body weight. Within treatment groups, injection-site lesions tended to be larger as the volume of microspheres injected increased. Consequently, the smallest dogs in each moxidectin SR treatment group had the smallest local reactions.

In general, analysis of kinetic data on serum moxidectin concentrations from this study supports the findings of long-term efficacy against challenge infection with *D immitis*. The terminal half-life of 50 days for the active ingredient after administration of moxidectin SR is much longer than the elimination half-life for native moxidectin in dogs, which is approximately 7 days. Thus, the slope and position of the terminal phase are interpreted to substantially represent slow release of moxidectin from the microspheres. This study did not determine the maximum duration of protection provided by moxidectin SR. If complete protection actually persists for periods of 7 months or longer, then a single properly timed injection of moxidectin SR could be used to provide chemoprophylaxis annually against heartworms in nearly all areas of the United States.[21] As indicated by the serial serum concentrations of moxidectin SR (Fig 1), *D immitis* larvae appear to be far more sensitive detectors of the compound than is chemical analysis of blood samples. Therefore, given the sensitivity of *D immitis* to moxidectin, additional testing to determine the ultimate potential of a sustained-release formulation to protect against infection with *D immitis* seems warranted.

The phenomenon of extended efficacy despite low serum concentrations may be attributable to partitioning of moxidectin between the tissue and plasma compartments. The degree to which serum and tissue concentrations of moxidectin correspond is unknown. However, for a related compound, ivermectin, drug concentrations detected in all tissues except brain far exceed those seen in blood, and the highest concentrations are found in fat.[22] Infective larvae of *D immitis* enter the tissues almost immediately, where they undergo 2 molts before invading the systemic venous circulation 70 to 85 days after inoculation.[23] Therefore, it is likely that postinfective larvae migrating through host tissues may be exposed to higher concentrations of moxidectin than are in plasma. This possibility justifies extending the interval between dose administration and challenge inoculation to determine the maximum duration of protection provided by a single dose of moxidectin SR at these concentrations.

McCall et al[24] documented that ivermectin and, to a lesser degree, milbemycin oxime provide substantial amounts of protection up to 4 months after exposure, a time when there would be a mixed population of late L4 and early fifth-stage juveniles. Efficacy against late-stage larvae is potentiated by continuing to administer the drug. The persistence of active ingredient in dogs treated with moxidectin SR calls for an investigation of this formulation's efficacy against preexisting infections with various stages of developing *D immitis* larvae.

The transition to sustained-release technology represents a fundamental change in the approach to chemoprophylaxis against infection with heartworms. Currently, the success of a preventative program hinges to a large degree on client compliance to adhere to a daily or monthly regimen of drug administration. Presumably, injectable moxidectin SR would be administered by veterinarians at a time judged to provide optimum coverage relative to the local season of transmission. Thus, aside from the motivation to visit the veterinarian once or twice a year for injection of moxidectin SR, the issue of client reliability would cease to be a factor in the successful prevention of infection with heartworms.

[a]Alder Ridge Farms Inc, Lakewood, Pa.
[b]Snap canine heartworm antigen test kit, IDEXX Laboratories, Westbrook, Me.
[c]Lot No. 96089901IL, Fort Dodge Animal Health, Princeton, NJ.
[d]Lot No. 96089902IL, Fort Dodge Animal Health, Princeton, NJ.
[e]R&R Research, Howard City, Mich.
[f]NCTC-135 medium, Sigma Chemical Co, St Louis, Mo.
[g]Iscoves modified Dulbecco's medium, Sigma Chemical Co, St Louis, Mo.
[h]ABC Laboratories, Columbia, Mo.
[i]M-2041 assay method, ABC Laboratories, Columbia, Mo.

Appendix

Constitution of 10% moxidectin microspheres for use in various treatments of dogs

10% Moxidectin microspheres (mg)	Vehicle (ml)	Volume (ml)†	Equivalent dose of active ingredient (ml/kg of body weight)
0	0*	30	0.0
175	14.8	15	0.06
500	14.5	15	0.17
1,500	13.5	15	0.50

*Dogs in the control group were given saline (0.9% NaCl) solution. †Each dog received an injection calculated on the basis of 0.05 ml/kg of body weight.

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