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Efficacy of an injectable, sustained-release formulation of moxidectin in preventing experimental heartworm infection in mongrel dogs challenged 12 months after administration

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Abstract

The objective of this study was to ascertain the ability of a single subcutaneous injection of a sustained-release (SR) formulation of moxidectin to protect dogs against challenge inoculation with infective *Dirofilaria immitis* larvae 364 days after administration. Twenty four purpose-bred adult mixed-breed dogs were grouped into three blocks of eight based on weight and sex. Saline solution (0.9% NaCl) or a moxidectin SR formulation at volumes designed to deliver 0.17 or 0.27 mg moxidectin/kg b.w. was injected subcutaneously on day 0. Throughout the post-treatment period, injection sites of all dogs were periodically examined visually and by palpation. Palpable swellings were characterized as to size, consistency and the presence of associated pain or erythema. On day 364, each dog was inoculated subcutaneously with 50 *D. immitis* L3. On days 510 and 511, dogs were euthanatized, and their hearts, lungs and thoracic cavities were inspected for the presence of adult heartworms. number, sex and viability of recovered heartworms were determined. The mean number of heartworms recovered from dogs that had received the saline control injection was 35.7. No heartworms were recovered from any dog treated with either 0.17 or 0.27 mg moxidectin/kg b.w. For variable periods of time following treatment, small (1–4 mm diameter), firm, subcutaneous swellings could be palpated at the injection sites of dogs treated with 0.17 or 0.27 mg moxidectin/kg b.w. These swellings contracted progressively and eventually disappeared except for the case of one animal treated with 0.27 mg/kg, in which the swelling persisted for the entire study period. At no time during the study was pain or erythema noted at the injection site of any dog, and no dog exhibited any adverse systemic reaction related to treatment. We conclude that under conditions pertaining in this study, a single subcutaneous injection of a moxidectin SR formulation at dosing rates of either 0.17 or 0.27 mg/kg b.w. can safely protect adult dogs against experimental challenge inoculation with infective heartworm larvae for a period of 12 months.

Keywords: Moxidectin; Mongrel dogs; Sustained-realease formulation; Heartworm

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1. Introduction

The heartworm, *Dirofilaria immitis*, causes a potentially debilitating and fatal cardiopulmonary disease in dogs and other susceptible animals, including domestic cats and ferrets. The life cycle includes an intermediate host phase completed within species of mosquito capable of serving as vectors for transmission of infective larvae during blood feeding from definitive mammalian hosts. Susceptible animals are at risk of infection with *D. immitis* whenever vector-competent mosquitoes are present and there is sufficient environmental heat to support development of the parasite within them.

Several drugs are effective for preventing heartworm infection. The technology of heartworm chemoprophylaxis has advanced from daily administration of diethylcarbamazine in the 1960s and 1970s (Kume, 1962; Kume et al., 1964, 1967) to monthly oral or topical administration of several macrolide endectocides in the 1980s and 1990s (Blair and Campbell, 1980; McCall et al., 1981, 1992; Paul et al., 1986; Grieve et al., 1991; King et al., 1992; McTier et al., 1992, 1999). All of the daily and monthly administered heartworm preventives are dispensed by veterinarians to pet owners who are responsible for maintaining the dosing regimen. Owner compliance with prescribed dosing regimens is problematic since failure to administer one or two scheduled daily doses of DEC (Filarbits®, Glaxo SmithKline, Philadelphia PA, USA; Filarbits Plus®, Pfizer, New York, NY, USA) or, depending upon the number of follow-up doses administered, one to two scheduled monthly doses of macrolide-based chemoprophylactics can diminish and eventually void protection.

The use of sustained-release formulations of macrolide endectocides, including moxidectin, provides an alternative to repetitive prophylactic dosing at shorter intervals in a number of livestock parasite systems (Miller et al., 1998; Munyua et al., 1998; Forbes et al., 1999; Cleale et al., 2004). It has been proposed that this approach could also be adapted to heartworm chemoprophylaxis (Miller et al., 1998). Previously, we demonstrated that an injectable, sustained-release formulation of the macrolide endectocide moxidectin (moxidectin SR), dosed to deliver 0.17 mg active ingredient per kg body weight, could protect dogs from challenge by experimental heartworm infection for 6 months (180 days) after administration (Lok et al., 2001, 2002). Because transmission of heartworm infection is a concern for 12 months per year in southern extremes of the continental United States (Knight and Lok, 1995; Watts et al., 2001), it was of particular interest to determine whether a single injection of a moxidectin SR formulation dosed to deliver 0.17 mg moxidectin/kg or higher could protect against challenge infection at 12 months. Recently, Genchi et al. (2002) reported evidence from a clinical field trial suggesting that single treatments with moxidectin SR can prevent heartworm infection for periods approaching an entire year in a region with seasonal transmission. In this study we present consistent evidence that moxidectin doses of 0.17 and 0.27 mg/kg in a sustained-release formulation which can protect dogs against experimental infection by inoculation of infective heartworm larvae 364 days later.

2. Materials and methods

2.1. Animals

The protocol for this experiment was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Twenty-four (11 male and 13 female) purpose-bred mongrel dogs were obtained from a commercial vendor.[1] The uneven sex ratio resulted from the removal of an unmanageable male dog and its replacement with a female during the acclimation period prior to beginning the study. These dogs had been reared since birth in a mosquito-free environment and had never been treated with a macrolide endectocide. While at the University of Pennsylvania, dogs were separated by sex and housed one or two dogs per run within a common room.

Pre-treatment conditioning of dogs, including testing for circulating heartworm microfilariae and adult worm antigen, was carried out as described previously (Lok et al., 2001). Two days prior to treatment (day 2), each dog was weighed, and the injection site on the left side of the neck immediately cranial to the scapula was clipped. Female and male dogs were ranked separately by descending weight.

[1] Alder Ridge Farms, Inc., Lakewood, PA.

Given the uneven sex ratio, the lightest female dog was treated as a male for purposes of group allocation. The resulting two 12-dog cohorts were stratified into four weight classes each containing three dogs. Male and female dogs from each of the four weight classes were assigned by random drawing to one of three groups of eight. Two groups contained four male and four female dogs, representing each of the four weight classes. The third group contained five female and three male dogs with equal representation of the four weight classes. Following this allocation, dogs were moved within the animal room so that the eight animals in each group occupied adjacent runs.

2.2. Procedures

A single suspension of moxidectin SR moxidectin microspheres[2] was prepared with a proprietary diluent[3] on the day of treatment (day 0). The dose for each moxidectin SR treated dog was calculated to deliver either 0.17 or 0.27 mg moxidectin/kg b.w. by injecting 0.05 ml/kg b.w. or 0.08 ml/kg b.w., respectively. The negative control dogs were injected with bacteriostatic saline (0.9% NaCl)[4] at a rate of 0.08 ml/kg b.w. Immediately prior to treatment, each group of eight dogs was assigned by random drawing to be injected with one of the two moxidectin SR doses or saline. An appropriate volume of the stock suspension of moxidectin SR or saline was injected subcutaneously at the clipped injection site on the neck of each dog. The principal investigators were blinded to the identity of the treatment groups. Veterinary technicians who assigned treatments to the dog groups and who administered the injections did not participate in any subsequent evaluations of the dogs, all of which were performed by the blinded investigators.

2.3. Challenge inoculation with D. immitis L3

Challenge inoculations were performed 12 months (364 days) post-treatment. The source of parasites and techniques for collection of L3 from laboratory-reared mosquitoes have been described previously (Lok et al., 2001). Two days prior to challenge, samples of venous blood were drawn from all dogs and examined for the presence of circulating microfilariae and adult heartworm antigen, also as described previously (Lok et al., 2001).

2.4. Antemortem observations

Dogs were observed 1, 2, 3, 4, 6, 8, 12 and 24 h following treatment for signs of adverse reactions. Each dog was observed in the same manner twice daily (morning and afternoon) on study days 1–7. For the remainder of the study, dogs were observed daily for signs of ill health.

Injection sites of all dogs were examined once on days 1 through 14 and then on days 21, 28, 35 and 41. For the remainder of the study, injection sites were examined once every 4 weeks. At each examination, tissues within a radius of approximately 6 cm surrounding the injection site were palpated. The size (diameter in mm) and consistency (soft, firm or hard) of palpable swellings, and presence of any attendant pain or erythema were evaluated.

2.5. Postmortem observations

Dogs were euthanatized with an overdose of pentobarbital sodium on days 510 (11 males) and 511 (13 females) following treatment. The final examination of each injection site was made just prior to necropsy. Procedures used to recover heartworms and characterize them by sex and viability status have been described previously (McCall et al., 1981; Lok et al., 2001).

3. Results

3.1. Parasitological findings

No heartworms were recovered from any of the dogs that had been treated with the moxidectin SR formulation dosed at the equivalent of either 0.17 or 0.27 mg moxidectin/kg b.w. (Table 1). By contrast, all dogs in the saline control group were infected with a mean of 35.7 adult heartworms per dog. This heartworm recovery rate represents approximately 71% of the L3 administered in the challenge

[2] Lot No. 367002, Fort dodge animal health, Princeton, NJ.
[3] Lot No. 368003, Fort dodge animal health, Princeton, NJ.
[4] Bacteriostatic Sodium Chloride Injection USP, Lot No. 382901, Fort dodge animal health,Princeton, NJ.

Table 1
Recovery of adult *D. immitis* from dogs inoculated with third-stage larvae 364 days after treatment with moxidectin SR

Treatment group	No. of dogs infected at necropsy[a]	Mean no. of heartworms recovered per dog		
		Males	Females	Total
Saline control	8	17.6	18.1	35.7 (14–52)[b]
0.17 mg/kg moxidectin SR	0	0	0	0
0.27 mg/kg moxidectin SR	0	0	0	0

[a] $N = 8$.
[b] Numbers in parentheses indicate the range.

inoculations. All of the recovered heartworms were alive, and the ratio of male to female parasites was 0.97. Tests for circulating microfilariae and adult heartworm antigen were negative in all dogs at the time of challenge inoculation.

3.2. Antemortem observations

At no time during the study did any dog exhibit an adverse systemic reaction that could be attributed to moxidectin SR treatment. Firm swellings, ranging in diameter from one to five mm, were initially detected at the injection sites of six of the eight dogs treated with 0.17 mg moxidectin/kg b.w. In three of these dogs the swellings slowly contracted and resolved completely by day 28 post-treatment. The other three dogs in this group had tiny, barely palpable swellings sporadically in the period between day 28 and day 313. No injection site swellings were detected after day 313 in the 0.17 mg moxidectin/kg b.w. treatment group. Seven of the eight dogs in the 0.27 mg moxidectin/kg b.w. treatment group developed palpable injection site swellings with diameters along their major axes ranging from 1 to 4 mm. Injection site swellings observed in the 0.27 mg moxidectin/kg b.w. treatment group resolved by day 13 in the case of one dog, by day 105 for three dogs, and by day 468 in two others. In only one dog did a palpable injection site swelling persist until the end of the study, and this measured approximately 1 mm × 3 mm 511 days post injection.

Over the course of the study, several incidents unrelated to treatment required medical intervention. In response to isolation of *Campylobacter* sp. from four dogs exhibiting bloody diarrhea, 10-day and 21-day courses of enrofloxicin,[5] 5 mg/kg b.w., once daily, were initiated in all dogs beginning on days 82 and 97. Superficial lacerations due to three episodes of fighting by dogs from the 0.27 mg moxidectin/kg b.w. treatment group (days 253, 362 and 372) and to accidental trauma to a foot pad of one dog in the saline control group (day 364) also were treated with standard courses of enrofloxicin. Beginning on day 461, one dog in the 0.17 mg moxidectin/kg b.w. treatment group was given a 14-day course of amoxicillin clavulanate[6] (13.75 mg/kg b.w. b.i.d.) after culture of *Staphylococcus* spp. from the aspirate of a swollen carpal joint.

4. Discussion

A previous evaluation of moxidectin SR heartworm chemoprophylaxis demonstrated 100% efficacy 6 months post-treatment at a dose of 0.17 mg moxidectin/kg b.w. (Lok et al., 2001). It is assumed that this formulation has the same minimum post-infection protection (retroactive efficacy) of 2 months as orally administered moxidectin (McCall et al., 1992; McTier et al., 1992). Thus, based on predicted seasonal limits of heartworm transmission (Slocombe, 1989; Knight and Lok, 1995), one properly timed annual injection of moxidectin SR delivering 0.17 mg moxidectin/kg b.w. should be sufficient to prevent infection in USA north of latitude 38°N and all of Canada. However, in the most southerly regions of USA, where heartworm transmission is longer or even continuous (Knight and Lok, 1995; Watts et al., 2001), a period of chemoprophylactic efficacy of 6–12 months is required. Neither laboratory efficacy trial conducted to date (Lok et al., 2002) determined a temporal endpoint for protection provided by this microsphere

[5] Baytril, Lot Nos. 169054, 169073 and 169070A, Bayer Corporation, Pharmaceutical Division,West Haven, CT.

[6] Clavamox, Lot no. NC0212, Pfizer animal health, Groton, CT.

formulation. More recently, however, a clinical field trial (Genchi et al., 2002) showed that protection for a period approaching 12 months after a single moxidectin SR injection is possible in a region where transmission occurs from May through September. In the present paper, we report consistent findings from a laboratory efficacy study.

In this study, a moxidectin SR formulation dosed to deliver 0.17 and 0.27 mg moxidectin/kg b.w. was 100% effective in protecting adult dogs from experimental challenge infection with *D. immitis* 12 months post-administration. The inherent differences between syringe inoculation and natural transmission of *D. immitis*, particularly the likely contribution of immunomodulators in vector saliva to establishment of infective larvae (Ribeiro, 1987; Cupp and Cupp, 1997), argue for caution in extrapolating these findings to veterinary practice. Furthermore, this study did not include young growing dogs experiencing weight changes during the study. However, field data indicating 12 months of protection under conditions of natural exposure in regions with seasonal transmission (Genchi et al., 2002) increase confidence in the formulation's efficacy.

It is noteworthy that although no dog in either of the treatment groups was infected, adult worms were recovered from all eight dogs in the saline control group. The average number of worms was nearly 36, with at least 14 recovered per control dog (Table 1). Since all dogs tested negative for heartworm infection at the inception of the study and at the time of the 50 L3 challenge inoculation, the recovery of 52 adult worms from one control dog probably represents a miscount of L3 in the inoculum.

As in previous evaluations of the commercially available moxidectin SR formulation delivering 0.17 of moxidectin per kg b.w., no adverse systemic responses to either treatment dose were observed in the present study. Local reactions at the injection site were limited to small swellings, detectable only after meticulous palpation. These focal swellings maintained a firm consistency, shrank to approximately the size of a grain of rice, and eventually disappeared in all but one instance.

The availability of 12 months sustained release heartworm chemoprophylaxis will have important implications for protecting dogs from this parasite in the southern parts of USA. Conscientious, timely administration of heartworm chemoprophylaxis is the weak link in maintaining a prevention program. Administration of a sustained release prophylactic by a veterinarian eliminates this uncertainty for the duration of the effective treatment interval. The prospect of providing protection would be significantly improved with a single injection of moxidectin SR that is effective for at least 14 months (2 months retroactive and 12 months proactive efficacy). Issues relating to the advocacy of uninterrupted monthly versus seasonally administered chemoprophylaxis become less important with this delivery technique because an entire year of protection is conveyed. However, strategic timing of administration is not entirely obviated. Although an injection can be given at any time of the year, the requirement to periodically retreat persists, and to achieve effective chemoprophylaxis with moxidectin SR, veterinarians must still motivate their clients to return for annual injections. Fortunately, heartworm transmission is limited to 6 or fewer months per year throughout much of USA, which provides a considerable buffer zone for timely continuation of protection. Therefore, in the event that a 12-month preventative becomes available in USA, injections should, to the extent possible, be scheduled during months when heartworm transmission has either ceased or is occurring at its lowest rate, generally November through March (Slocombe, 1989; Knight and Lok, 1995; Watts et al., 2001).

The method of providing heartworm chemoprophylaxis also influences testing guidelines for surveillance. Prior to first initiating prophylaxis with any drug, infection status should be determined in all dogs that may have been exposed to infective mosquitoes at least 7 months previously. Because of the long prepatent period, optimal testing is best achieved with two antigen tests at 6-month intervals. However, if 12-month injectable moxidectin SR chemoprophylaxis becomes available in USA, retesting beyond these two initial tests should conform to guidelines of the American Heartworm Society (American Heartworm Society, 2003) after confirming that the program is intact the first year. Good client education and communication are essential for ensuring protection by motivating dog owners to adhere to a prescribed dosage schedule.

To summarize, in this study, a sustained release formulation of moxidectin impregnated microspheres,

injected subcutaneously into dogs at a dose of 0.17 mg moxidectin/kg b.w. and 0.27 mg moxidectin/kg b.w., provided complete protection against experimental challenge inoculation of heartworm (*D. immitis*) infective larvae 12 months after treatment.

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