Dirofilaria immitis and D. repens in dog and cat and human infections

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Safety and efficacy of selamectin in dogs with *Dirofilaria immitis* infection

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In both laboratory (McTier et al., 1998, 2000) and field studies (Clemence et al., 2000; Boy et al., 2000), selamectin has proven to be highly effective in the prevention of Dirofilaria immitis (heartworm) infections in dogs and cats following monthly prophylactic administrations at a minimum dose rate of 6 mg/kg. In studies in which administration occurred 45 days (simulated delayed treatment) or 60 days (simulated delayed or missed treatment) after inoculation with infective third-stage larvae (L3), a single treatment with selamectin was completely effective (McTier et al., 2000). It has also been demonstrated that no adverse effects are likely if selamectin is inadvertently administered to animals with existing infections of adult D. immitis (Novotny et al., 2000) In heartworm endemic areas, there are several potential scenarios for compliance failure in heartworm prevention, which a clinician may encounter.

These include dogs that have been exposed to infection for various periods prior to initiation of their heartworm prophylaxis, situations of missed monthly dosing, and circumstances in which dogs with patent infections and various degrees of microfilaraemia are presented for treatment. The series of studies presented here was designed to assess the performance of selamectin in 3 situations, which hitherto had not been investigated. The studies included an assessment of the safety and efficacy of selamectin in heartworm-positive dogs with high microfilaraemia (study A); the microfilaricidal and adulticidal efficacy of selamectin after long-term monthly administration to dogs with patent D. immitis infections (study B); and the “reachback” or clinical prophylactic effect in dogs inoculated with heartworm L3 3 months prior to initiation of long-term monthly prophylaxis (study C).

Materials and methods

Treatments

Selamectin in the commercial formulation\(^a\) was administered as a unit dose to provide a minimum dose rate of 6 mg/kg of body weight. The placebo (vehicle only) was administered at the rate of 0.05 ml/kg of body weight to provide an equivalent volume.

Treatments were applied to the skin at a single site on each dog’s back at the base of the neck and cranial to the scapulae. Treatments were administered at 30-day intervals beginning on study day 0.

Animals

Equal numbers of colony-bred male and female Beagles were used. The initial age and weight ranges for the dogs were 5 to 7 months and 6.45 to 15.4 kg, respectively. Dogs were housed individually in mosquito-proof indoor pens inside purpose-built accommodations, with controlled temperature and ventilation systems. They were fed once daily with an appropriate quantity of maintenance diet and water was supplied ad libitum. Animals were main-

\(^a\) Stronghold\(^\circledR\)/Revolution\(^\circledR\), Pfizer Inc., New York, New York, USA.
tained with due regard for their welfare and in accordance with applicable legislation and guidelines.

Parasite challenge

For dogs in studies A and B, mature adult *D. immitis* worms aged approximately 10 to 12 months were obtained from donor animals having experimentally induced infections. Twenty worms (10 males, 10 females) were introduced surgically into the cranial vena cava via the external jugular vein while the dogs were under general anaesthesia. For study C, *D. immitis* L3 were obtained from experimentally infected *Aedes aegypti*, and each dog was inoculated with approximately 50 L3 by subcutaneous injection into the inguinal region 3 months prior to initiation of treatment.

*Dirofilaria immitis* antigen testing and microfilarial counting

Dogs were tested for the presence of *D. immitis* antigen by using a commercial test kit. A modified Knott concentration technique was used to count microfilariae.

Adult worm counts

After euthanasia, dogs in studies B and C were necropsied for recovery of adult *D. immitis*. The pleural and peritoneal cavities were examined for adult worms, and the cranial and caudal vena cavae were clamped before removal of the heart and lungs (and liver in study C). The pre-cava, right atrium, right ventricle, and pulmonary arteries (and caudal vena cava as far as the liver in study C) were dissected and the worms removed, recorded as male or female, recorded as alive or dead, and fixed in formalin.

Design

Study A. Safety and efficacy in dogs with high microfilarial counts

Eighteen dogs (9 males, 9 females) that tested negative for heartworm antigen and microfilariae were inoculated with 20 adult *D. immitis* on day –222. Sixteen dogs (8 males, 8 females) with the highest *D. immitis* microfilarial counts (mean, >10,000 mf/ml on day –5) were selected, weighed, and randomly allocated to treatment with selamectin or a placebo. Treatments were administered on days 0, 30, and 60. Clinical observations were made prior to each treatment, at 4 and 8 hours after treatment, and once daily for the next 7 days. General health observations were made daily on all other days throughout the study. Blood was collected by venipuncture for microfilarial counting on the day of treatment (days 0, 30, and 60) and on days 1, 7, and 14 after each of the 3 treatments.

Study B. Efficacy of long-term treatment against adult *D. immitis* and circulating microfilariae

Eighteen dogs (9 males, 9 females), with negative results for blood microfilariae and heartworm antigen on day

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b DiroChek® Canine Heartworm Antigen Test Kit, Synbiotics Corporation, San Diego, California, USA.
32, were each inoculated with 20 adult *D. immitis* worms on day 30. On day 3, 16 dogs (8 males, 8 females) with the highest levels of microfilariae and antigen were selected, weighed, and randomly allocated to treatment with selamectin or a placebo. A total of 18 monthly treatments were administered on days 0, 31, 60, 90, 122, 152, 182, 213, 243, 273, 304, 334, 364, 395, 425, 455, 486, and 516. Clinical observations were made prior to each treatment, and then within 10 minutes, and at 2, 4, 8, and 24 hours after treatment. General health observations were made daily on all other days throughout the study. Blood was collected by venipuncture for microfilarial counting and antigen testing approximately every 30 days. All dogs were euthanized on day 546 for necropsy and recovery of adult *D. immitis*.

### Study C. Long-term monthly prophylaxis beginning at 3 months after heartworm inoculation (“Reachback” efficacy)

Seventeen dogs that had tested negative for microfilariae and *D. immitis* antigen on day –92 were each inoculated with approximately 50 *D. immitis* L3 on day –90. On day –3, blood samples were obtained for microfilariae counting and antigen testing. On day –1, 16 dogs were selected and allocated to treatment with selamectin or a placebo (8 dogs/treatment). Monthly treatments were administered beginning on day 0 (90 days after infection), and continuing thereafter for 11 months (total of 12 monthly treatments). Clinical observations were made immediately prior to each treatment, and then at 4, 8, and 24 hours after treatment. General health observations were made daily on all other days throughout the study. Blood samples for microfilarial counts and antigen tests were collected at approximately 30-day intervals and the dogs were euthanized on day 363 (15 months after inoculation) for necropsy and recovery of adult *D. immitis*.

### Data analysis

Parasite counts were log transformed \(\ln(x+1)\) prior to analysis by using a repeated-measures model. *A priori* contrasts among least squares means of the log transformed data were used to assess the treatment differences on each counting day at the 5% level of significance \((P \leq 0.05)\). Geometric mean parasite counts for each treatment were calculated from least squares means of the log transformed data, and these means were used to estimate percentage reductions in parasite burden at each time point for those animals treated with selamectin, compared with those receiving the placebo, using the following formula:

\[
\text{(Geometric mean count for placebo) - (geometric mean count for selamectin)} / \text{(Geometric mean count for placebo)} \times 100 = \% \text{ reduction}
\]

### Results

#### Study A. Safety and efficacy in dogs with high microfilarial counts

There were no adverse clinical observations related either directly to treat-
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ment or to the effects of treatment on parasites within heartworm-positive dogs during the study. Geometric mean microfilarial counts for placebo-treated dogs on days 0, 1, 30, 60, and 74 were 9,882, 7,117, 3,780, 6,322, and 2,523 mf/ml, respectively, compared with selamectin-treated dogs, which had counts on the same days of 11,568, 10,462, 459, 606, and 223 mf/ml, respectively (Table 1). Thus, the microfilarial count for the selamectin-treated dogs gradually decreased during the month after the first treatment (87.5% reduction on day 30), compared with the placebo-treated controls, and generally remained at approximately that value for the remainder of the study. The microfilarial counts were significantly ($P \leq 0.05$) lower for selamectin-treated dogs, compared with placebo-treated dogs, on days 30, 31, 67, and 74.

### Study B. Efficacy of long-term treatment against adult *D. immitis* and circulating microfilariae

At necropsy, the geometric mean adult *D. immitis* counts were 10 for selamectin-treated dogs and 15 for placebo-treated dogs (controls). There was no significant difference between the treatments, even though the reduction in worm count for the selamectin-treated dogs was 36.4%. All of the live worms recovered from the selamectin-treated dogs and placebo-treated dogs were normal in motility. Only 1 live worm recovered from the control dogs was abnormal in appearance (i.e., 1 female worm was opaque white), whereas 6 of 8 selamectin-treated dogs had abnormal appearing worms. Twenty-one of the 51 worms from selamectin-treated dogs were

| Table 1. Geometric mean counts of *Dirofilaria immitis* microfilariae following monthly treatment of dogs with an initial high microfilaremia. Treatment began 222 days after experimentally acquired infection with adult worms. |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Treatment* | Day 0 | Day 1 | Day 7 | Day 14 | Day 30 | Day 31 | Day 37 | Day 44 | Day 60 | Day 61 | Day 67 | Day 74 |
| Placebo (n=8) | 9,882 | 7,117 | 6,776 | 917 | 3,780 | 3,653 | 4,401 | 4,938 | 6,322 | 5,228 | 3,878 | 2,523 |
| Selamectin (n=8) | 11,568 | 10,462 | 2,620 | 432 | 459 | 496 | 654 | 796 | 606 | 458 | 336 | 223 |
| % reduction** | 0.0% | 0.0% | 61.3% | 52.9% | 87.9% | 86.4% | 85.1% | 83.9% | 90.4% | 91.2% | 91.5% | 91.2% |
| $P$-value† | NS | NS | NS | NS | ≤0.05 | ≤0.05 | NS | NS | NS | NS | ≤0.05 | ≤0.05 |

* Dogs were treated on days 0, 30, and 60.
** For selamectin treatment compared with placebo.
† Significance of difference between treatments.
NS = Not significant.
abnormal (i.e., opaque white with or without a darkened anterior end). All dogs in both treatments remained positive for heartworm antigen throughout the study, except for 1 selamectin-treated dog that tested negative on 1 occasion (day 483).

On day −3 (prior to the first treatment), the mean microfilarial counts were 382 and 315 mf/ml for the placebo- and selamectin-treatment groups, respectively (Fig. 1). For dogs treated with the placebo, the count increased to >1,000 mf/ml on day 28 (1 month after the first dose) and to >3,000 mf/ml by day 59. The count was >10,000 mf/ml from day 119 through the end of the study, except on days 362 (12 months) and 392 when counts were 7,284 and 5,207 mf/ml respectively. For dogs treated with selamectin, the mean microfilarial count showed no increase from day 3 to day 28 (348 mf/ml), and then declined to reach 0 at day 180. From day 180 through the end of the study (day 544, after 18 monthly treatments), the count remained 0 except on days 271, 301, and 332 when there was a mean count of 1 mf/ml. From day 59 to the end of the study, selamectin-treated dogs had significantly ($P \leq 0.05$) fewer microfilariae than dogs treated with the placebo. The reductions in geometric mean microfilarial count for dogs treated with selamectin, compared with those treated with the placebo, were 66.9% on day 28, 95.8% on day 59, 99.7% on day 90, 99.9% on day 119, and approximately 100% from day 150 to study completion.

No adverse reactions attributed to selamectin treatment were observed in this study.

**Study B**

![Graph](attachment:graph.png)

* Treatment differences were significant ($P \leq 0.05$) from day 59 through completion (day 544).

Fig. 1. Geometric mean counts of *Dirofilaria immitis* microfilariae in dogs treated monthly, beginning 30 days after experimentally induced infection with adult worms.
Table 2. Geometric mean counts of adult *D. immitis* at necropsy following monthly treatment that began 90 days after inoculation with L₃ in dogs.

**Study C**

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Live worms</th>
<th>Dead worms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=8)</td>
<td>19.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Selamectin (n=8)</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>% reduction**</td>
<td>98.5%</td>
<td>100%</td>
</tr>
<tr>
<td>*-value†</td>
<td>≤0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Dogs were treated on days 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 330.
** For selamectin treatment compared with placebo.
† Significance of difference between treatments.
NS = Not significant.

**Study C. Long-term monthly prophylaxis beginning at 3 months after heartworm inoculation ("Reachback" efficacy)**

At necropsy (15 months after inoculation), the geometric mean counts of live adult *D. immitis* from placebo- and selamectin-treated dogs were 19.8 and 0.3, respectively (Table 2). There was a significant (*P ≤ 0.05*) difference between the treatments, and the reduction in the heartworm burden for selamectin-treated animals, compared with that for the control group, was 98.5%. Dead heartworms were recovered at necropsy from the placebo-treated dogs only, but there was no significant difference between treatments for the number of dead heartworms. Adult *D. immitis* antigen tests were negative for all 16 dogs (both placebo- and selamectin-treated) from day –94 until day 57 (Fig. 2). From day 118 through day 361, the percentage of antigen-positive dogs for the placebo-treatment group remained above 85%, whereas for selamectin-treated dogs, the percentage never exceeded 58% and decreased over time, with 12.5% of dogs antigen-positive on day 361. Microfilarial counts were negative for all 16 dogs until day 118, and then only dogs treated with the placebo had positive counts. The selamectin-treated dogs remained negative throughout the study (Table 3).

**Discussion**

Results of the first study presented here (study A) found that no adverse effects were observed when selamectin was administered monthly for 3 months to dogs with high microfilaria (mean, >10,000 mf/ml) prior to the first application. Furthermore, just 3 treatments with selamectin at monthly intervals was shown to have a substantial microfilaricidal effect, as demonstrated by reductions in microfilaria count of >90%, compared with those for the control group (study A). The microfilaricidal activity was con-
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confirmed in dogs with lower initial levels of microfilariae (study B), where a reduction of 95% in microfilarial counts was observed by the third month after commencing monthly treatment and 100% by the sixth month. It is generally considered that if microfilaremic dogs are treated with an effective microfilaricide, there is an increased risk of adverse effects occurring during the first 4 to 8 hours after administration (Knigth, 1998). Such reactions following the rapid death of large numbers of microfilariae can occur with microfilaremias of at least 5,000 mf/ml, but are more usually associated with burdens greater than 10,000 mf/ml. Reactions include lethargy, inappetance, ptyalism, vomiting, tachycardia, pallor, and acute circulatory collapse (Knigth, 1998). From previous studies with other macrolides,

Table 3. Geometric mean counts of D. immitis microfilariae following monthly treatment that began 90 days after inoculation with L3 in dogs.

Study C

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</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>80</td>
<td>986</td>
<td>1,205</td>
<td>3,325</td>
<td>3,355</td>
<td>13,131</td>
<td>2,554</td>
<td>2,219</td>
</tr>
<tr>
<td>Selamectin (n=8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>% reduction**</td>
<td>-</td>
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Fig. 2. Percentages of dogs positive for D. immitis antigen in dogs treated monthly, beginning 90 days after inoculation with (L3) larvae.

* Dogs were treated on days 0, 30, 60, 90, 120,150, 180, 210, 240, 270, 300, and 330.
** For selamectin treatment compared with placebo.
notably ivermectin (20-50 mg/kg, ie, higher than prophylactic dosages) and milbemycin oxime, (prophylactic dosage) treatment-induced reductions in the levels of circulating microfilariae in dogs with patent infections have been demonstrated (Courtney et al., 1998). Milbemycin oxime causes a more rapid reduction in microfilaremia than ivermectin (Courtney et al., 1998). Thus, it is possible to see adverse effects due to dead microfilariae, particularly following the use of milbemycin oxime (prophylactic/microfilaricidal dosage), and it is recommended that microfilaricidal dogs be carefully monitored for at least 8 hours after treatment with microfilaricidal dosages of these drugs (Knight, 1998). It has been shown previously that selamectin can be safely administered to dogs with mature adult heartworms and low levels of circulating microfilariae (Novotny et al., 2000).

The efficacy of selamectin in gradually reducing levels of microfilariae (90-100%) over time offers benefits in terms of reducing transmission potential within a population. This effect indicates the potential for selamectin to be used following adulticidal therapy to eliminate a residual microfilaria in dogs with patent heartworm infections. In common with other macrolides, such as ivermectin and milbemycin, when selamectin is administered to dogs with patent infections, the dogs may become amicrofilaric resulting in “occult” infections. Hence, in a dog with an unknown history or when it is possible that there has been exposure to infection, particularly in young dogs or strays, it is important to establish the heartworm status of the dog prior to commencement of a prophylactic regimen. However, if for some reason this is omitted, the majority of infected amicrofilaric dogs should be identifiable by using an adult antigen test.

It is not known at present whether microfilarial counts will increase following cessation of monthly administration of selamectin, as has been reported for milbemycin and ivermectin (Courtney et al., 1998). The other macrolides have been shown to effect embryogenesis in female heartworms (Lok and Knight, 1995); however, at this time, the effects of selamectin on adult worms, particularly female worms, have not been fully characterized.

Historically, as a class of compounds, the macrolides have not been considered to have activity against mature adult heartworms. In study B, when treatment was initiated against mature adult heartworms (11 months old) and treatment was administered monthly for 18 consecutive months, there were 36.4% fewer adult heartworms present in selamectin-treated dogs than in the controls, however, there was no statistical difference between the worm counts in the 2 treatment groups. In addition, many of the live selamectin-treated worms were abnormal in appearance. It is possible that additional monthly treatments with selamectin would increase the efficacy against adult heartworms. When ivermectin was administered monthly for 16 consecutive months to dogs harbouring mature adult heartworms, efficacy was 56.3% (McCall et al., 1998), and when treatment was administered for 29 months, efficacy improved to 94.9% (McCall et al., 1998).
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2001). It has also been suggested that if an effective regimen could be devised, ivermectin might offer some clinical advantages in heartworm-infected dogs, because the slow killing effect may reduce the risk of adverse effects (particularly those associated with pulmonary thromboembolism) from dead worms or worm fragments, compared with the risk of using adulticidal agents (melarsomine, thiacetarsamide), which have a much more rapid killing effect on adult heartworms, which leads to the death of some dogs (McCall et al., 1998).

These studies demonstrated that selamectin, when administered as 12 monthly treatments beginning 3 months after L3 infection (reachback), is also highly effective in preventing the maturation of heartworms, with a reduction of 98.5%.

Previously it has been shown that monthly prophylaxis for 13 months with ivermectin or milbemycin, beginning 3 months after L3 infection, significantly reduces the number of adult worms that develop by 97.7% and 96.8% for ivermectin and milbemycin, respectively (McCall et al., 1996). It has also been found that monthly prophylaxis for 12 months beginning 4 months after L3–induced infection leads to 95.1% and 41.4% reductions in maturation to adult worms for ivermectin and milbemycin, respectively (McCall et al., 1996). The effects of long-term monthly prophylactic treatment with selamectin, commencing 6 months after inoculation, is currently under investigation, and results will be forthcoming.

Conclusions

Selamectin is microfilaricidal but kills microfilariae relatively slowly, even in dogs with high microfilaremia. This gradual reduction of microfilariae virtually eliminates, or at least greatly minimizes potential adverse reactions due to large numbers of dead or dying microfilariae. Selamectin appears to have an adverse effect on mature adult heartworms, but the effect is gradual during long-term monthly prophylaxis. This slow killing effect on adult heartworms should minimize the potentially severe adverse effects seen with a rapid kill of adult heartworms. In addition, selamectin has a 3-month reachback effect that is similar to the other macrolide preventives. Thus, selamectin has demonstrated exceptional safety in heartworm-positive dogs and, together with robust reachback activity, increases the confidence with which this product can be used in the fight against heartworm disease in dogs.

Acknowledgements

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References


Clemence RG, Sarasola P, Genchi C, Smith DG, Shanks DJ, Jernigan AD, Rowan TG, 2000. Efficacy of selamectin in the prevention of adult heartworm (Dirofilaria immitis) infection in


McCall JW, Roberts RE, Supakorndej N, Mansour A, Dzimianski MD, McCall SD, 2001. Further evidence of clinical prophylactic (reachback) and adulticidal activity of monthly administra-


