# Safety of Simparica Trio® (sarolaner, pyrantel, moxidectin) in heartworm-infected dogs

**Sheerin Mathur** ( sheerin.mathur@zoetis.com )
Zoetis (United States)
**Phyllis B Malpas**
Zoetis (United States)
**Sean Mahabir**
Zoetis (United States)
**Joseph Boucher**
Zoetis (United States)
**Aleah Pullins**
Zoetis (United States)
**Genevieve Gagnon**
Zoetis (United States)
**Tom L. McTier**
Zoetis (United States)
**Steven Maeder**
Zoetis (United States)

**Research Article**

**Keywords:** Simparica Trio, Moxidectin, Safety, Heartworm Disease, Dirofilaria immitis, Dogs, Microfilaremia, Pharmacokinetics, PK

**Posted Date:** January 6th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-2439526/v1



**Additional Declarations:** No competing interests reported.

**Version of Record:** A version of this preprint was published at Parasites & Vectors on March 31st, 2023. See the published version at https://doi.org/10.1186/s13071-023-05700-9.

## Abstract

# Background

Assessment of the safety of heartworm preventatives in dogs with pre-existing, patent, heartworm (*Dirofilaria immitis*) infections is necessary because rapid adult worm and microfilarial death can lead to severe clinical complications, including thromboembolism and anaphylactic shock in dogs. The aim of this study was to determine the clinical safety of Simparica Trio® (sarolaner, pyrantel, moxidectin) in heartworm-infected dogs and the degree of microfilaricidal and adulticidal activity of 3 consecutive monthly treatments of Simparica Trio.

# Methods

Twenty-four laboratory Beagle dogs were implanted with 10 male and 10 female *D. immitis* (ZoeKY isolate) and once infection was patent, were randomized equally among 3 groups to receive negative control, 1X, or 3X of the maximum recommended label dose of Simparica Trio. Dogs in the treated groups received Simparica Trio on days 0, 28 and 56 as whole tablets to achieve the maximum recommended label dose 2.4 mg/kg of sarolaner + 10 mg/kg pyrantel + 48 µg/kg (0.048 mg/kg) moxidectin. In-life assessments included body weight, physical examinations, clinical observations, daily general health observations, a quantitative estimate of food consumption, and blood collections for pharmacokinetic (PK) analysis, microfilariae (MF) counts, and *D. immitis* antigen testing. At the end of the study the heart, lungs, and pleural and peritoneal cavities were examined for adult *D. immitis* worms.

# Results

Simparica Trio was generally well-tolerated. Emesis occurred at low frequency in all groups including control. Abnormal stool occurred occasionally in 1X and 3X groups throughout the 3-month study. Fever (> 104°F/40°C) was recorded in one 1X and one 3X dog one day after the first dose and resolved by the following day. No severe hypersensitivity reactions occurred. The mean number of circulating microfilariae (MF) counts in the control group increased from 12,000/mL at study start (Day 0) to > 20,000/mL at Day 28 and remained above 20,000/mL for the duration of the study. The least squares mean of circulating MF were reduced by 69.8% on Day 1 and 97.4% on Day 7 for the 1X group and remained at > 99% lower than the control group for the remainder of the study. Similarly, least squares mean of circulating MF were reduced by 85.3% on Day 1 and 93.9% on Day 7 for the 3X group and remained > 98% lower than the control group for the remainder of the study. At the end of the study, the mean number of implanted adult worms recovered was fewer than 10 per sex in all groups with 90%, 85%, and 75% of live adult heartworms recovered in control, 1X, and 3X treatment groups, respectively. Low numbers of dead adult worms were recovered in 1X and 3X, with none in control. Following each dose, the moxidectin and sarolaner AUC and $C_{max}$ had close to dose proportional increases.

# Conclusions

This study demonstrated that Simparica Trio (sarolaner, pyrantel, moxidectin) was well-tolerated when administered to heartworm-positive dogs at 1X, and 3X the maximum recommended dose at 28-day intervals for 3 consecutive months. Simparica Trio significantly reduced microfilaria counts in both treatment groups, without significant clinical consequences. At the doses administered, Simparica Trio had minor adulticidal activity, but resulted in no clinical sequelae.

# Background

Simparica Trio (Zoetis) is the first combination endectocide containing an isoxazoline (sarolaner), moxidectin, and pyrantel that is licensed in the United States that prevents heartworm disease (*Dirofilaria immitis*) and treats and controls fleas, ticks, and intestinal nematodes. Simparica Trio is a chewable, flavored tablet with a minimum label dosage of 1.2 mg/kg sarolaner, 5 mg/kg pyrantel (as pamoate salt) and 24 µg/kg moxidectin. Other licensed combination products contain the macrocylcic lactone (MLs) ivermectin, moxidectin, or milbemycin oxime for canine heartworm disease prevention. Among MLs, moxidectin has some unique attributes such as high potency, long half-life, wide therapeutic index and versatility in formulation that could help in overcoming heartworm drug resistance (Savadelis et al. [1]). The dose of moxidectin included in Simparica Trio was optimized to increase the effectiveness against known drug-resistant heartworm isolates (McTier et al. [2]).

Macrocyclic lactones (ivermectin, selamectin, milbemycin oxime, moxidectin) target the L3 and L4 infective stages of *D. immitis* in the subcutaneous tissues. While this class of drug is most potent against the L3 and L4 stages, MLs at the doses used for HW prevention can also affect both microfilariae (MF) and adult worms (Bowman et al. [3]). These effects vary with the specific ML, the dose of the ML, and the number of consecutive doses administered for monthly products, or the length of time after treatment for the extended-release injectable products. MLs in combination with doxycycline can have additional adulticidal effects over time (Savadelis et al. [4]; Alberigi et al. [5]). Furthermore, MLs are considered generally safe for use in dogs harboring existing adult HW infections with circulating MF unlike the previously licensed daily oral heartworm preventives containing diethylcarbamazine (Powers et al. [6]). This attribute of MLs makes preventing new heartworm infections during melarsomine (Immiticide® / Diroban®) adulticidal heartworm treatment possible. The American Heartworm Society (AHS) recommends that “A macrocyclic lactone preventive should be administered for 2 months prior to administering melarsomine to reduce new infections and eliminate existing susceptible larvae” [7]. If dogs are still positive for microfilariae by Day 120 of treatment, the AHS recommends administering a microfilaricide. Currently only one moxidectin-containing product is approved as a heartworm microfilaricide (Advantage Multi® Bayer), however all MLs using label and extra-label doses at varying frequencies of dosages have demonstrated microfilaricidal effects (Bowman et al. [3]).

It is necessary to evaluate any new heartworm disease preventive products for safety in HW-positive dogs before product approval to ensure its safe use in heartworm-positive dogs in the general population. The purpose of the current investigation was to evaluate the safety of the maximum therapeutic dose of Simparica Trio (2.4 mg/kg of sarolaner + 10 mg/kg pyrantel + 48 µg/kg (0.048 mg/kg) moxidectin) in dogs with laboratory-induced adult *D. immitis* infections and with circulating MF.

# Methods

# Animals

A total of 24 purpose-bred laboratory Beagle dogs, including 12 males and 12 females approximately 10 months of age, were enrolled in the study. Dogs were housed individually in environmentally controlled indoor runs with ad libitum access to food and water.

Approximately 4 months prior to study initiation, dogs were surgically transplanted with 10 male and 10 female *D. immitis* worms (ZoeKY isolate) a known ML susceptible isolate (Rawlings et al. [8]). Prior to the study initiation, infection status was verified by a *D. immitis* antigen test (DiroCHEK® Heartworm Antigen Test Kit, Zoetis, Parsippany,NJ) and blood samples were verified to contain > 500 circulating microfilariae (MF) per mL by modified Knott's method. No animal had previous exposure to a macrocyclic lactone.

# Study Design

This study was conducted in compliance with requirements of US FDA GLP regulations (21CFR Part 58) [9]. The study aligned with recommendations of the "VICH GL 43 (Target Animal Safety) – Pharmaceuticals [10]". To control bias, treatment allocation was not disclosed to any person responsible for conducting subjective observations. This study was also conducted in compliance with the Animal Welfare Act of 1966 (and subsequent amendments including the Animal Welfare Acts of 1970, 1976, and 1985) for the care of animals. The study procedures, and care and use of animals were reviewed and approved in advance of the study by test facility Institutional Animal Care and Use Committee.

The 24 dogs were randomized to treatments (8 per group) according to split-plot design within room, with sex as the whole plot factor and treatment as the split-plot factor. Animals were blocked by sex and microfilariae (MF) count and randomized within blocks of size 3 and assigned to treatment groups: negative control (empty capsule), 1X and 3X of the maximum recommended label dose of Simparica Trio. Dogs in the treated groups received Simparica Trio on days 0, 28 and 56 administered as whole tablets (Table 1). The recommended minimum dosage for the product is 1.2 mg/kg sarolaner + 5 mg/kg pyrantel + 24 µg/kg (0.024 mg/kg) moxidectin. However, per VICH GL 43, the margin of safety is assessed based on multiples of the maximum recommended therapeutic dose (MRTD) which is the dose intended for administration to the lightest-weight dog in the widest dose band. For Simparica Trio, the MRTD is 2.4 mg/kg of sarolaner + 10 mg/kg pyrantel + 48 µg/kg (0.048 mg/kg) moxidectin. The doses were based on the most recent individual body weight. Because point dosing of the calculated MRTD is not possible with whole tablets, doses were rounded up using the smallest tablet size.

In-life assessments included weekly body weight measurements, veterinary physical examinations, veterinary clinical observations, technician-led daily general health observations, and a daily quantitative estimate of food consumption. Blood collections for pharmacokinetic (PK) analysis were performed pre-dose, and 2, 8, 24, 72, 168, 336, 504, and 672 hours after each dose. Moxidectin, sarolaner and pyrantel plasma concentrations were measured using validated LC-MS/MS methods. Blood was also collected for heartworm testing throughout the study as follows: MF counts on Days − 14, -8, 0 (pre-dose), 1, 3, 7, 14, 21, 28 (pre-dose), 35, 56 (pre-dose), 63, and 83; and *D. immitis* antigen testing Day − 14 and pre-dose on days 28, 56, 83.

Table 1
Margin of safety study design of Simparica Trio in heartworm-infected (*Dirofilaria immitis*) Beagle dogs

| Treatment Group | Treatment | Dose Level | # Dogs (M/F) | Dosing Days | Necropsy Day |
|---|---|---|---|---|---|
| Control [a] | Placebo | 0 | 8 (4/4) | 0, 28, and 56 | 84 |
| 1X [b] | Sarolaner | 2.4 mg/kg | 8 (4/4) | | |
| | Pyrantel | 10 mg/kg | | | |
| | Moxidectin | 48 µg/kg | | | |
| 3X [b] | Sarolaner | 7.2 mg/kg | 8 (4/4) | | |
| | Pyrantel | 30 mg/kg | | | |
| | Moxidectin | 144 µg/kg | | | |

[a] Empty HPMC capsules for oral administration.

[b] Maximum dose from the dose banding.

Insert Table 1

Dogs were humanely euthanized after an intravenous injection of heparin, to facilitate parasite recovery, via an approved pentobarbital euthanasia solution on Day 84. After euthanasia, the pleural and peritoneal cavities were examined for adult *D. immitis* worms, and the posterior and anterior venae cavae were clamped before removal of the heart and lungs. The pre-cava, right atrium, right ventricle and pulmonary arteries (including those coursing through the lungs) were dissected and examined for worms. The worms from each dog were counted, identified as adult male or female, and classified as either dead or alive based on motility and appearance (Holmes et al. [11]).

# Data Analysis

Observational data (general health observations, clinical observations, and physical exams) were summarized with frequency distributions by treatment and timepoint. Body temperature, body weight, weekly average food consumption, adult heartworm counts, and MF counts were summarized with descriptive statistics. Body weight, weekly average food consumption, and MF counts were statistically analyzed using a general linear mixed model for repeated measures including fixed effects of treatment, time, sex, and all interactions among those effects. The random effects included room, block within room and sex, the interaction of block and treatment within room and sex (animal term), and error. MF counts were natural log transformed prior to modeling and back-transformed least squares means and corresponding confidence intervals were reported by treatment and timepoint.

For sarolaner and moxidectin, plasma concentration means and 90% confidence intervals were calculated for each treatment and timepoint using the statistical model described above. A natural log transformation was applied to plasma concentration data prior to modeling and back-transformed least squares means and corresponding confidence intervals were reported by treatment and timepoint. For sarolaner and moxidectin

estimates of the pharmacokinetic (PK) parameters $C_{max}$, $t_{max}$, $AUC_{0-672h}$, and $t_{1/2}$ were made using non-compartmental methodology. For pyrantel $C_{max}$, $t_{max}$ and $AUC_{0-24h}$ were estimated. The means and confidence intervals for the PK parameters were calculated using a similar general linear mixed model for repeated measures as described above with dose period replacing time in the statistical model. AUC and $C_{max}$ were natural log transformed prior to modeling and back-transformed least squares means and corresponding confidence intervals were reported by treatment.

For the repeated measures models described above, if the treatment by sex by time interaction was significant at the 5% level of significance, then no further testing was done. If the sex by treatment by time interaction was not significant and the treatment by sex or time interaction was significant at the 10% level of significance, then treatment LS means were calculated and pairwise comparisons between control and treatments were performed at the unadjusted 10% level of significance, within each sex if the treatment by sex interactions was significant ($P \leq 0.10$), or within timepoint if the treatment by time interaction was significant ($P \leq 0.10$) or timepoint if the treatment by time interaction was significant ($P \leq 0.10$). If none of the interactions involving treatment were significant and the treatment main effect was significant at the 10% level of significance, then the treatment LS means were calculated and pairwise comparisons between control and treatments were performed at the unadjusted 10% level of significance. If no treatment related effects were significant, no treatment comparisons were performed.

## Results

# Clinical Signs

Clinical signs observed are summarized by treatment group in Table 2. Fever occurred in 2 treated animals (one dog in the 1X group, one dog in the 3X group) on Day 1 of the study. Both observations of fever resolved by Day 2 without medical intervention. Emesis was observed at low frequency in all treatment groups, including dogs in the control group, throughout the study. Minor sporadic decreases in weekly food consumption were observed in dogs in the 1X and 3X treatment groups; but there were no correlating changes in body weight. No hypersensitivity reactions (defined as anaphylaxis, shock, collapse, respiratory distress, depression, or fever) were identified in any dog at any timepoint. Abnormal stool (soft stool or mucoid diarrhea or hemorrhagic colitis) was observed in 2 dogs in control group, 4 dogs in 1X group and 7 in 3X group. This observation appeared to be treatment related. In all cases the effects observed were transient and condition resolved without treatment.

Table 2

Clinical signs by treatment group (control group, 1X treatment group; 3X treatment group): Number of animals affected and total number of observations Days 0–84

| Clinical Sign | Control | | 1X | | 3X | |
|---|---|---|---|---|---|---|
| | # Dogs | # Obs | # Dogs | # Obs | # Dogs | # Obs |
| Abnormal Stools | 2 | 3 | 4 | 23 | 7 | 23 |
| Decreased Appetite | 0 | 0 | 4 | 5 | 5 | 6 |
| Fever (> 104°F/40°C) | 0 | 0 | 1 | 1 | 1 | 1 |
| Emesis | 3 | 3 | 2 | 3 | 4 | 7 |

Insert Table 2

# Microfilarial Counts

MF Counts were performed throughout the study (Table 3). Mean MF counts (least square means) were significantly lower in both treated groups compared with the negative control at every timepoint after Day 0 ($P \leq 0.016$). The mean number of circulating microfilariae (MF) counts in the control group increased from 12,000/mL at study start (Day 0) through Day 28 to > 20,000/mL and remained above 20,000/mL for the remaining duration of the study. The least squares mean of circulating MF were reduced by 69.8% on Day 1 and 97.4% on Day 7 for the 1X group and continued to remain > 99% lower relative to the control group for the remainder of the study. Similarly, least squares mean of circulating MF were reduced by 85.3% on Day 1 and 93.9% on Day 7 for the 3X group and continued to remain > 98% lower relative to the control group for the remainder of the study (Fig. 1).

Table 3

Least square means microfilariae count (counts/mL) for post-treatment timepoints

| Study Day | 1 | 3 | 7 | 14 | 21 | 28 | 35 | 56 | 63 | 83 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 12289 | 20149 | 24440 | 27462 | 25695 | 28659 | 26342 | 23717 | 22519 | 21622 |
| 1x | 3711* | 2503* | 644* | 179* | 212* | 104* | 80* | 147* | 265* | 24* |
| % Reduction | 69.8 | 87.6 | 97.4 | 99.3 | 99.2 | 99.6 | 99.7 | 99.4 | 98.8 | 99.9 |
| 3x | 1803* | 1833* | 1494* | 359* | 221* | 146* | 110* | 112* | 205* | 3* |
| % Reduction | 85.3 | 90.9 | 93.9 | 98.7 | 99.1 | 99.5 | 99.6 | 99.5 | 99.1 | > 99.9 |
| *Significant at 2-sided alpha = 0.10 ($P$< = 0.10). | | | | | | | | | | |

Insert Table 3

# Adult Worm Counts

At the end of the study, the mean number of implanted adult worms recovered was less than 10 per sex in all groups with 90%, 85%, and 75% of live adult heartworms recovered in control, 1X, and 3X treatment groups, respectively (Table 4). No dead adult worms were found in the control group. Four (3 female and 1 male) dead adult worms were found in 2 dogs in the 1X group. Three (1 female and 2 male) dead adult worms were found in 3 dogs in the 3X group. All dogs in all treatment groups remained positive for heartworm antigen throughout the study.

Table 4
Recovery of total, live, and dead heartworm counts on Day 84

| Animal | Treatment | Total Number of Worms Recovered | Number of Adult Males | | Number of Adult Females | |
|---|---|---|---|---|---|---|
| | | | Live | Dead | Live | Dead |
| 3128025 (M) | Control | 13 | 4 | 0 | 9 | 0 |
| 3128972 (M) | Control | 19 | 10 | 0 | 9 | 0 |
| 6533043 (M) | Control | 19 | 10 | 0 | 9 | 0 |
| 6533116 (M) | Control | 18 | 8 | 0 | 10 | 0 |
| 6532535 (F) | Control | 19 | 9 | 0 | 10 | 0 |
| 6532578 (F) | Control | 16 | 9 | 0 | 7 | 0 |
| 6532675 (F) | Control | 18 | 8 | 0 | 10 | 0 |
| 6532918 (F) | Control | 20 | 10 | 0 | 10 | 0 |
| 6532152 (M) | 1X | 15 | 8 | 0 | 7 | 0 |
| 6532187 (M) | 1X | 17 | 6 | 0 | 11 | 0 |
| 6532217 (M) | 1X | 18 | 11 | 0 | 7 | 0 |
| 6532241 (M) | 1X | 17 | 8 | 0 | 9 | 0 |
| 3124127 (F) | 1X | 19 | 9 | 0 | 10 | 0 |
| 3124763 (F) | 1X | 18 | 9 | 0 | 9 | 0 |
| 6532501 (F) | 1X | 15 | 7 | 1 | 5 | 2 |
| 6532519 (F) | 1X | 18 | 9 | 0 | 8 | 1 |
| 3127380 (M) | 3X | 9 | 1 | 0 | 8 | 0 |
| 6532560 (M) | 3X | 15 | 9 | 0 | 5 | 1 |

| Animal | Treatment | Total Number of Worms Recovered | Number of Adult Males | | Number of Adult Females | |
|---|---|---|---|---|---|---|
| | | | Live | Dead | Live | Dead |
| 6532641 (M) | 3X | 10 | 4 | 0 | 6 | 0 |
| 6533027 (M) | 3X | 17 | 8 | 0 | 9 | 0 |
| 6532250 (F) | 3X | 19 | 10 | 0 | 9 | 0 |
| 6532764 (F) | 3X | 17 | 8 | 1 | 8 | 0 |
| 6533159 (F) | 3X | 18 | 8 | 0 | 10 | 0 |
| 6533167 (F) | 3X | 17 | 8 | 1 | 8 | 0 |

Insert Table 4

# Pharmacokinetic Data

As expected, the pharmacokinetic analysis demonstrated the LS mean plasma concentration in the 3X group was approximately 3 times higher than the 1X group for both moxidectin (Fig. 2) and sarolaner. Pyrantel had very low bioavailability and very high variability, but plasma concentrations did show an increased level with dose. Following each dose, the moxidectin and sarolaner AUC and $C_{max}$ had expected dose-related increases. Although not formally tested, the increases appear to be close to dose proportional. Moxidectin and sarolaner show accumulation in dose-normalized $AUC_{0-672h}$. The accumulation ratios based on $AUC_{0-672h}$ from dose 1 to dose 3 for moxidectin and sarolaner were approximately 1.7.

## Discussion

In this study, the safety of a novel combination of sarolaner, pyrantel and moxidectin (Simparica Trio, Zoetis) in dogs experimentally infected with adult *D. immitis* and with circulating microfilariae was evaluated. When used per label, a single dose of Simparica Trio in the commercially available formulation delivers a dose of moxidectin between 24–48 µg/kg. Treatment of heartworm-positive dogs with 1X and 3X the maximum label dose of Simparica Trio was well-tolerated with treatment-related observations limited to occasional abnormal stools (e.g. soft stool, diarrhea), decreased appetite, and, rarely, fever.

# Microfilaricidal Activity

Moxidectin administration resulted in significant reduction of MF counts at both doses of 48 µg/kg (1X) and 144 µg/kg (3X) in dogs infected with adult *D. immitis* (Fig. 1). The mean MF count in the control group increased from 12,000/mL at study start (Day 0) to > 20,000/mL at Day 28 and remained above 20,000/mL for the duration of the study. The mean number of circulating MF were reduced by > 90% on Day 3 for the 144

µg/kg (3X) group and by Day 7 for the 48 µg/kg (1X) group compared with controls and remained > 98% compared to the control group for the remainder of the study.

The only relevant clinical sign observed was fever in two dogs approximately 24 hours after dosing with Simparica Trio. A single 1X dog had a body temperature of 104.1°F (40.0°C) on the day after treatment. This dog's MF count was 22,000/mL just prior to treatment with Simparica Trio, falling to 3900/mL the following day which corresponded to a > 80% reduction. A second dog, in the 3X group, was described as "subdued" and had a body temperature of 105.4°F (40.7°C) on the day after treatment. This dog had a pre-treatment body temperature of 104.5°F (40.3°C) possibly indicating that the dog was reacting to its high microfilaremia prior to dosing with Simparica Trio. This dog's MF count was 27,000/mL just prior to treatment and fell to 1500/mL the following day, a 93% reduction. Both dogs cleared all MF by 1 (1X) and 3 (3X) weeks after the first treatment. The fever exhibited by these dogs could be attributed to the immune response to the dying microfilariae after they had been exposed to moxidectin. It is hypothesized that the ML activity on glutamate gated chloride ion channels present in the excretory/secretory pore of microfilariae, inhibits the secretion of immunomodulatory molecules thereby allowing the host immune response to clear microfilariae (Wolstenhome et al. [12]).

By the end of the study after 3 treatments with Simparica Trio, 3 dogs in 1X treatment group and 2 dogs in 3X treatment group had dropped to zero MF count. The two dogs discussed above were the only 2 dogs with complete clearance of MF prior to the last data collection point. Although other dogs had higher MF counts pre-dose, they did not exhibit fever after dosing with Simparica Trio. This may be due to individual variability in immune response, or because the mild fever was of such short a duration that it was not present at the times of recording.

Overall, despite a rapid decline in MF counts in both treatment groups, which has also been observed with other ML-containing heartworm disease preventives, no severe or anaphylactic-like adverse reactions akin to those observed with the previously used daily heartworm preventive drug, diethylcarbamazine, occurred (Powers et al. [6]).

# Adulticidal Activity

The rapid killing of adult heartworms in dogs could result in severe pulmonary thromboembolism or death (Ames et al. [13]). There were no clinical observations attributable to rapid adulticidal activity in this study. Occasionally, as a result of surgical transplant procedure, worms may be injured and subsequently die and eventually be absorbed by the body, and this was indicated by recovering fewer than 20 worms (live or dead) from some animals in the control group. The recovery of the majority of adult male and female live worms in both 1X (48 µg/kg) and 3X (144 µg/kg) groups, however, indicates there was no rapid kill of worms and there were no clinical signs consistent with severe pulmonary thromboembolism: cough, dyspnea, exercise intolerance, collapse, hemoptysis, death. There were more dead worms in the Simparica Trio treated groups than the control group indicating that moxidectin may have had some minor adulticidal activity, but with no clinical sequelae.

## Conclusions

This study demonstrated that dogs that had been previously infected with ML susceptible adult *D. immitis* (ZoeKY isolate) and subsequently treated with Simparica Trio at 1X and 3X the maximum recommended label

dose, 3 times at 28-day intervals, was well-tolerated. Simparica Trio caused a rapid but clinically benign reduction of microfilariae counts in both treatment groups with minimal effects on adult worms.

# Declarations


### Acknowledgements

The authors would like to thank the veterinarians, technicians, and their staff, for their participation in this study, as well as Zoetis Veterinary Medicine Research and Development leadership for their support in study funding.

### Funding

Zoetis provided all funding for this study


### Availability of data and materials

Not applicable

### Authors' contributions

Sheerin Mathur - Overall responsibility (single point of control) for the technical conduct of the study, including overseeing the design, conduct, documentation, analysis, interpretation, and reporting of results.

Phyllis Malpas – Test Facility Manager, ensured adequate resources were available for conduct of the study.

Sean Mahabir - Randomization(s), data summary and analysis.

Joseph Boucher – Pharmacokinetic contributing scientist.

Aleah Pullins and Tom McTier- Parasitologist implanted heartworms in dogs, examined the heart, lungs, pleural and peritoneal cavities for adult *D. immitis* worms.

Genevieve Gagnon- Progress the study according to the plan; liaise with / coordinate the activities of partner groups, contributing scientists; data review and compilation; archiving.

Steven Maeder- Zoetis program leader.

All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The study protocol was reviewed and approved by the relevant test facility Animal Care and Use Committee prior to implementation.

### Consent for publication

Not applicable

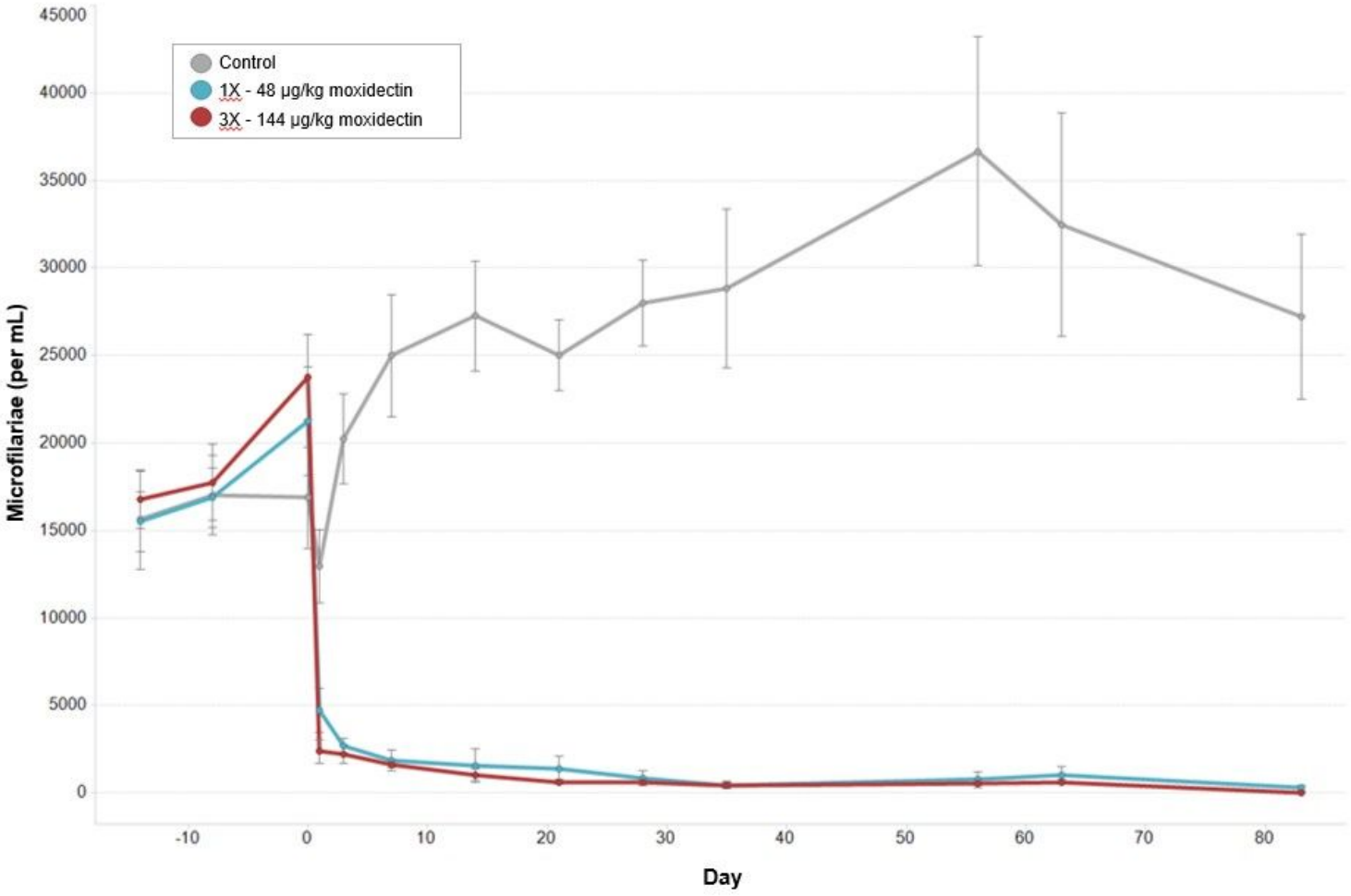
### Competing interests

All authors were current employees of Zoetis at the time the study was performed.

# References


1. Savadelis MD, McTier TL, Kryda K, Maeder SJ, Woods DJ. Moxidectin: heartworm disease prevention in dogs in the face of emerging macrocyclic lactone resistance. Parasites Vectors 2022; 15, 82.
2. McTier TL, Six RH, Chapin S, Kryda K, Mahabir, SP, Woods DJ, Maeder SJ. Preventive efficacy of moxidectin at various doses and dosage regimens against macrocyclic lactone-resistant heartworm (*Dirofilaria immitis*) strains in dogs. Parasites Vectors 2019;12:444.
3. Bowman DD, Mannella C. Macrocyclic lactones and *Dirofilaria immitis* microfilariae. Top Companion Anim Med. 2011;26(4):160-172.
4. Savadelis MD, Ohmes CM, Hostetler JA, Settje TL, Zolynas R, Dzimianski MT, et al. Assessment of parasitological findings in heartworm-infected beagles treated with Advantage Multi® for dogs (10% imidacloprid + 2.5% moxidectin) and doxycycline. Parasites Vectors 2017;10:245.
5. Alberigi B, Fernandes JI, Paiva JP, Mendes-de-Almeida F, Knackfuss F, Merlo A, et al. Efficacy of semi-annual therapy of an extended-release injectable moxidectin suspension and oral doxycycline in *Dirofilaria immitis* naturally infected dogs. Parasites Vectors 2020;13:503.
6. Powers KG, Parbuoni EL, Furrow RD. *Dirofilaria immitis*: Adverse reactions associated with diethylcarbamazine therapy in microfilaremic dogs, in Otto GF (ed): Proceedings of the Heartworm Symposium ’80. Dallas, Texas 23-24 February, 1980, pp 108-116, 1981.
7. American Heartworm Society. Current canine guidelines for the prevention, diagnosis, and management of heartworm (*Dirofilaria immitis*) infection in dogs. 2020. https://d3ft8sckhnqim2.cloudfront.net/images/pdf/AHS_Canine_Guidelines_11_13_20.pdf?1605556516. Accessed 9 November 2022.
8. Rawlings CA, McCall JA. Surgical transplantation of adult *Dirofilaria immitis* to study heartworm infection and disease in dogs. Am J Vet Res. 1985;46(1):221–4.
9. Code of Federal Regulations Title 21, Part §58 Good Laboratory Practice for Nonclinical Laboratory studies. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58 (current as of Jul 2022).
10. VICH Guideline GL 43 “Target Animal Safety - Pharmaceuticals: Target Animal Safety for Veterinary Pharmaceutical Products”, July (2008) http://www.vichsec.org/.
11. Holmes RA, McCall JW, Prasse KW. Thiacetarsamide in dogs with *Dirofilaria immitis*: influence of decreased liver function on drug efficacy. Am J Vet Res. 1986;47:1341–4.
12. Wolstenhome AJ, Maclean MJ, Coates R, McCoy CJ, Reaves BJ. How do macrocyclic lactones kill filarial nematode larvae? Invert Neurosci. 2016;16:7.
13. Ames MK, Atkins CE. Treatment of dogs with severe heartworm disease. Vet Parasitol. 2020;283:109131.

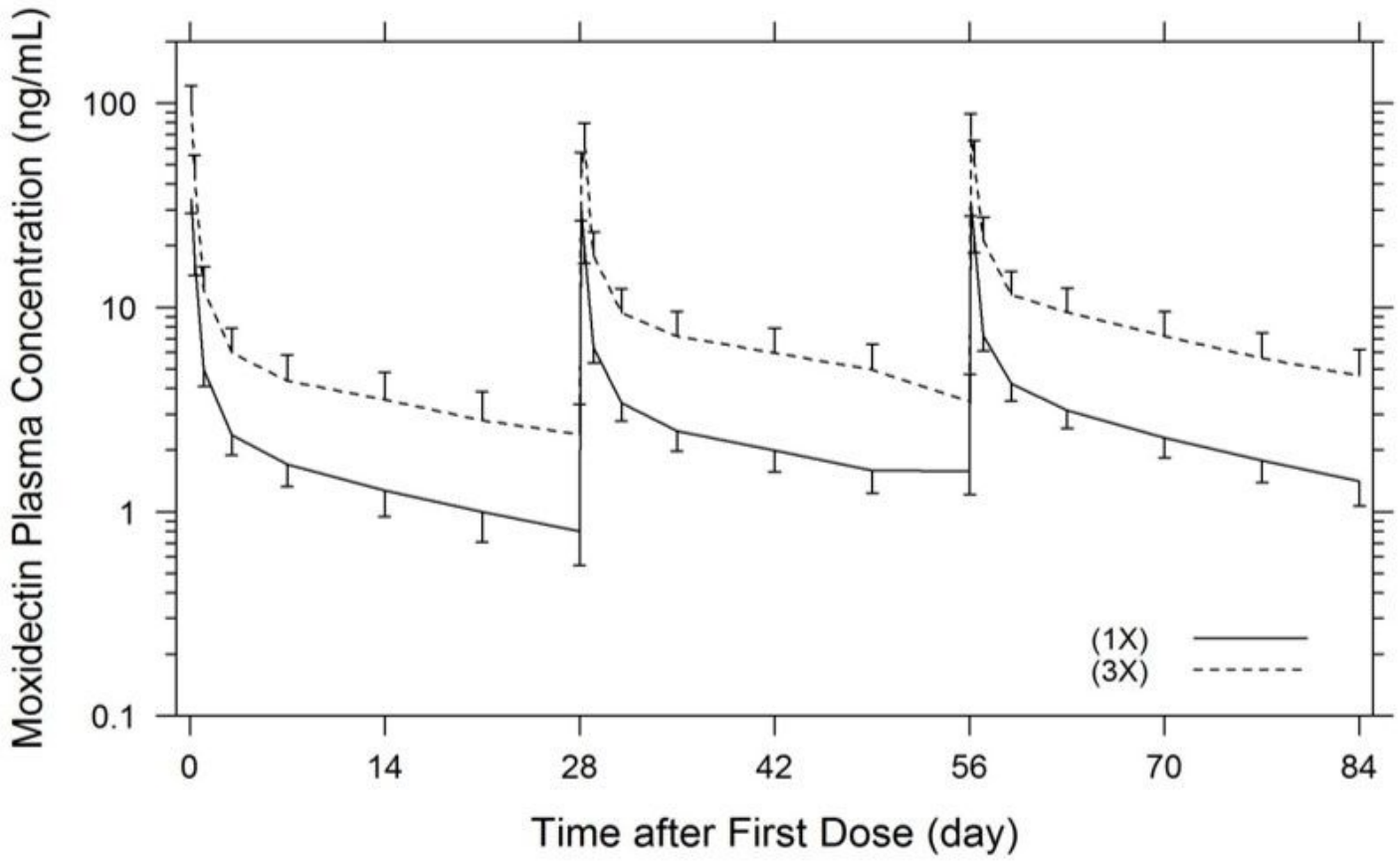

# Figures




## Figure 1

Mean microfilariae (MF) count/mL for each treatment by modified Knott's method. All study dogs were evaluated for MF on Days -14, -8, 0 (pre-dosing), 1, 3, 7, 14, 21, 28 (pre-dosing), 35, 56 (pre-dosing), 63, and 83. A significant reduction ($P$<0.10) in MF was observed in both 1X and 3X treatment groups at all time points following dosing[A1]

[A1]Check to make sure this aligns with figure titles see page 10 of style guide




## Figure 2

LS Mean (+/- 90% CI) moxidectin plasma concentration following each of 3 monthly (28-day interval) doses of Simparica Trio