Date of Approval: December 23, 2021

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-521

Simparica TRIO®

(sarolaner, moxidectin, and pyrantel chewable tablets)

Chewable Tablet

Dogs

This supplement provides for the addition of the indications, "for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks" and "for the treatment and control of L4 and immature adult *Ancylostoma caninum"*

Sponsored by:

Zoetis, Inc.

Executive Summary

Simparica TRIO® (sarolaner, moxidectin, and pyrantel chewable tablets) is approved for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks and for the treatment and control of immature (L4) and immature adult *Ancylostoma caninum*. The drug is given orally once a month and is approved for dogs and puppies 8 weeks of age and older and weighing 2.8 pounds or greater.

B. burgdorferi are spirochete bacteria that can cause Lyme disease in dogs. The bacteria are transmitted to a dog through the bite of infected *I. scapularis* ticks, also called black-legged ticks. Simparica TRIO® was previously shown to be effective against *I. scapularis* ticks for one month in dogs. The studies that support this supplemental approval showed that the drug kills *I. scapularis* ticks on treated dogs before they can transmit *B. burgdorferi*.

A. caninum is the main cause of canine hookworm disease in most tropical and subtropical areas of the world. The parasite eggs are passed in a dog's feces 15 to 20 days after infection, and transmission occurs when other dogs ingest the infective larvae from the environment. The third larval stage (L3) is the infective stage, and they molt to the fourth larval stage (L4) and then into adult parasites inside the dog. *A. caninum* can also be transmitted to puppies through the colostrum or milk of infected nursing dogs. Simparica TRIO® was previously shown to be effective against adult *A. caninum* in dogs. The studies that support this supplemental approval showed that the drug is also effective against L4 and immature adult *A. caninum*.

Simparica TRIO® is already approved to prevent heartworm disease caused by *Dirofilaria immitis* and to treat and control roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and hookworm (*Ancylostoma caninum* and *Uncinaria stenocephala*) infections. Simparica TRIO® is also already approved to kill adult fleas (*Ctenocephalides felis*) and to treat and prevent flea infestations and the treatment and control several types of tick infestations for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater.

Proprietary Name	Established Name	Application Type and Number	Sponsor
Simparica TRIO®	sarolaner, moxidectin, and pyrantel chewable tablets	New Animal Drug Application (NADA) 141-521	Zoetis Inc.

Simparica TRIO® contains three active ingredients: sarolaner, moxidectin, and pyrantel pamoate.

Sarolaner is an ectoparasiticide belonging to the isoxazoline class. The drug inhibits gamma-aminobutyric acid (GABA)-gated chloride channels in fleas and ticks. Chloride ions are blocked from crossing cell membranes, which results in uncontrolled neuromuscular activity in fleas and ticks, causing their death. Sarolaner is selectively toxic to fleas and ticks because their GABA receptors are more sensitive to the drug than mammalian GABA receptors.

Moxidectin is a parasiticide belonging to the macrocyclic lactone class. It is effective against larval stages of *D. immitis*, the parasite that causes heartworm disease. Moxidectin binds to and activates chloride channels in the heartworm larvae, which causes increased permeability and an influx of chloride ions. This results in flaccid paralysis and death of the parasites.

Pyrantel pamoate is a nematocide belonging to the tetrahydropyrimidine class. The drug is effective against intestinal nematodes and acts as a depolarizing, neuromuscular blocking agent in the parasites. This causes paralysis of the nematodes, allowing the dog to naturally expel the parasites in its feces.

Safety and Effectiveness

Preventing B. burgdorferi Infections by Killing I. scapularis Ticks

The sponsor conducted two laboratory studies to show that Simparica TRIO® protects dogs from infection with *B. burgdorferi* by killing infected *I. scapularis* ticks on the dogs before they can transmit the bacteria. In each study, dogs were administered either Simparica TRIO® or a placebo on Day 0 and then infested with approximately 50 unfed, wild-caught, adult *I. scapularis* ticks on Day 28. The ticks had a *B. burgdorferi* infection rate of 75 to 80%. The ticks were counted and removed on Day 33.

Blood samples were collected from all dogs on multiple days throughout the study, from 7 to 8 days before treatment to 104 days after treatment. The samples were both qualitatively and quantitatively tested for *B. burgdorferi* antibodies. Skin biopsies from each dog were collected on Day 104 from the heaviest areas of tick attachment, as marked on Day 33, and were quantitatively tested for *B. burgdorferi* by polymerase chain reaction (PCR) testing.

In both studies, Simparica TRIO® was 100% effective at killing *I. scapularis* ticks for 33 days in treated dogs, while dogs in the control group remained infested with live ticks. Simparica TRIO® was also 90 to 100% effective at preventing *B. burgdorferi* infections as a direct result of killing *I. scapularis* ticks. In one study, nine of the ten dogs treated with Simparica TRIO® remained seronegative for *B. burgdorferi* throughout the study and the bacteria were not detected by PCR testing of the skin biopsies. In the other study, all ten dogs treated with Simparica TRIO® remained seronegative for *B. burgdorferi* throughout the study and the bacteria were not detected by PCR testing of the skin biopsies. In contrast, all dogs in the control group in both studies became seropositive for *B. burgdorferi* and the bacteria were detected by PCR testing of the skin biopsies. No adverse reactions were seen in either study.

Treating and Controlling Immature (L4) A. caninum

The sponsor conducted two laboratory studies to show that Simparica TRIO® is effective against the immature stage (L4) of *A. caninum*. In each study, the dogs were inoculated orally with approximately 200 (±50) infective larvae (L3) of *A. caninum* on Day -7 and then administered either Simparica TRIO® or a placebo on Day 0 (7 days is enough time for the L3 parasites to molt to L4). One week after treatment (on Days 7 and 8), the dogs were necropsied and the parasites were counted. In both studies, dogs in the control group had adequate *A. caninum* infections, and compared to the control group, dogs in the treated group had significantly lower parasite counts. Simparica TRIO® was greater than 98% effective against L4 *A. caninum*. No adverse reactions were seen in either study.

Treating and Controlling Immature Adult A. caninum
The sponsor conducted two laboratory studies to show that Simparica TRIO® is effective against immature adult *A. caninum*. In each study, the dogs were inoculated orally with approximately 200 (±50) infective larvae (L3) of *A. caninum* on Day -11 and then administered either Simparica TRIO® or a placebo on Day 0 (11 days is enough time for the L3 parasites to molt to L4 and then into immature adults). One week after treatment (on Day 7), the dogs were necropsied and the parasites were counted. In both studies, dogs in the control group had adequate *A. caninum* infections, and compared to the control group, dogs in the treated group had significantly lower parasite counts. Simparica TRIO® was greater than 99% effective against immature adult *A. caninum*. No adverse reactions were seen in either study.

The FOI Summary for the original approval of Simparica TRIO®, dated February 27, 2020, contains a summary of target animal safety studies for dogs.

Precautions
Sarolaner, one of the active ingredients in Simparica TRIO®, is in the isoxazoline class. This class has been associated with neurologic adverse reactions including tremors, ataxia, and seizures in some dogs. Seizures have been reported even in dogs without a history of seizures. The drug should be used with caution in dogs with a history of seizures or other neurologic disorders.

Before the first administration of Simparica TRIO®, dogs should be tested for existing heartworm infections. Heartworm-positive dogs should be treated with an adulticide to remove adult heartworms. Simparica TRIO® is not effective against adult heartworms.

The safe use of Simparica TRIO® has not been evaluated in breeding, pregnant, or lactating dogs.

Conclusions
Based on the data submitted by the sponsor for the approval of Simparica TRIO®, FDA determined that the drug is safe and effective when used according to the labeling.

Table of Contents

I. GENERAL INFORMATION

A. File Number

NADA 141-521

B. Sponsor

Zoetis Inc.
333 Portage St.
Kalamazoo, MI 49007

Drug Labeler Code: 054771

C. Proprietary Name

Simparica TRIO®

D. Drug Product Established Name

Sarolaner, moxidectin, and pyrantel chewable tablets

E. Pharmacological Category

Antiparasitic

F. Dosage Form

Chewable Tablet

G. Amount of Active Ingredient

Each chewable tablet contains:
3.0 mg sarolaner / 0.06 mg moxidectin / 12.5 mg pyrantel (as pamoate salt)
6.0 mg sarolaner / 0.12 mg moxidectin / 25.0 mg pyrantel (as pamoate salt)
12.0 mg sarolaner / 0.24 mg moxidectin / 50.0 mg pyrantel (as pamoate salt)
24.0 mg sarolaner / 0.48 mg moxidectin / 100 mg pyrantel (as pamoate salt)
48.0 mg sarolaner / 0.96 mg moxidectin / 200 mg pyrantel (as pamoate salt)
72.0 mg sarolaner / 1.44 mg moxidectin / 300 mg pyrantel (as pamoate salt)

H. How Supplied

Simparica TRIO® is available in six sizes, in color-coded packages of 1, 3, or 6 flavored chewable tablets.

I. Dispensing Status

Prescription (Rx)

J. Dosage Regimen

Simparica TRIO® is given orally, once a month, at the recommended minimum dose of 0.54 mg.lb (1.2 mg/kg) sarolaner, 0.011 mg/lb (24 µg/kg) moxidectin, and 2.27 mg/lb (5 mg/kg) pyrantel (as pamoate salt).

Body Weight (lbs)	Sarolaner per Tablet (mg)	Moxidectin per Tablet (mg)	Pyrantel per Tablet (mg)	Number of Tablets Administered	Color Coding on Carton
2.8 to 5.5	3.0	0.06	12.5	One	Gold
5.6 to 11.0	6.0	0.12	25.0	One	Purple
11.1 to 22.0	12.0	0.24	50.0	One	Caramel
22.1 to 44.0	24.0	0.48	100.0	One	Blue
44.1 to 88.0	48.0	0.96	200.0	One	Green
88.1 to 132.0	72.0	1.44	300.0	One	Dark Brown
> 132.0	Administer the appropriate combination of tablets				

K. Route of Administration

Oral

L. Species/Class

Dog

M. Indication

Simparica TRIO® is indicated for the prevention of heartworm disease caused by *Dirofilaria immitis* and for the treatment and control of roundworm (immature adult and adult *Toxocara canis* and adult *Toxascaris leonina*) and hookworm (L4, immature adult, and adult *Ancylostoma caninum* and adult *Uncinaria stenocephala*) infections. Simparica TRIO® kills adult fleas *(Ctenocephalides felis)* and is indicated for the treatment and prevention of flea infestations, and the treatment and control of tick infestations with *Amblyomma americanum* (lone star tick), *Amblyomma maculatum* (Gulf Coast tick), *Dermacentor variabilis* (American dog tick), *Ixodes scapularis* (black-legged tick), and *Rhipicephalus sanguineus* (brown dog tick) for one month in dogs and puppies 8 weeks of age and older, and weighing 2.8 pounds or greater. Simparica TRIO® is indicated for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks.

N. Effect of Supplement

This supplement provides for the addition of the indications, for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks and for the treatment and control of L4 and immature adult *Ancylostoma caninum*.

II. EFFECTIVENESS

The effectiveness of Simparica TRIO® was demonstrated in six well-controlled laboratory studies described below. No treatment-related adverse reactions were reported in any of the 53 dogs administered the labeled dose in these studies. These studies demonstrated that Simparica TRIO® is effective in preventing *B. burgdorferi* infections by killing *I. scapularis* ticks on the dogs before they could transmit the infection and is effective in treatment and control of L4 and immature adult *A. caninum* in dogs. The Freedom of Information Summary for the original approval of NADA 141-521, dated February 27, 2020, contains a summary of the data that demonstrated that Simparica TRIO® is effective against *I. scapularis* ticks for 35 days and for the treatment and control of adult hookworm *(A. caninum)*.

A. Dosage Characterization

This supplemental approval does not change the previously approved 0.54 mg/lb (1.2 mg/kg) sarolaner, 0.011 mg/lb (24 µg/kg) moxidectin, and 2.27 mg/lb (5 mg/kg) pyrantel (as pamoate salt) doses, given orally once a month. The Freedom of Information (FOI) Summary for the original approval of NADA 141-521, dated February 27, 2020, contains the dosage characterization information for dogs.

B. Substantial Evidence

For the prevention of Borrelia burgdorferi infections by killing I. scapularis ticks:

1. Laboratory Dose Confirmation Study (Study No. A166C-US-20-B31)

   Title: Evaluation of the Ability of Simparica TRIO® to Prevent the Transmission of *Borrelia burgdorferi* from Infected *Ixodes scapularis* to Dogs.

   Study Dates: November 30, 2020 to June 29, 2021

   Study Location: Waverly, NY

   Study Design:

   Objective: To evaluate the ability of Simparica TRIO® to protect dogs against *B. burgdorferi* infections from wild caught *I. scapularis* ticks by killing the ticks before transmission may occur.

   Study Animals: Twenty (20) Beagle dogs (15 male and 5 female), 10-11 months of age, and 3.3 to 5.5 kg body weight.

   Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with Good Clinical Practices (GCPs). The study included two treatment groups of 10 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin and 5 mg/kg pyrantel (as pamoate salt). The treatment was administered on Day 0. Each dog was infested with approximately 50 unfed, wild-caught, adult *I. scapularis* ticks (approximately equal numbers of males

and females) on Day 28. The ticks had a *B. burgdorferi* infection rate of 80%. The ticks were counted and removed on Day 33.

Table II.1. Study Design for Study A166C-US-20-B31

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group[2]	Day of Infestation	Day of Tick Count	Days of Blood Collection	Day of Skin Biopsy Collection
T01	Placebo	N/A	Day 0	9	28	33	-7, 27, 49, 63, 77, 91, and 104	104
T02	Simparica TRIO® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	10	28	33	-7, 27, 49, 63, 77, 91, and 104	104

[1] Minimum Dosage
[2] One dog in T01 was removed from the study after *B. burgdorferi*-positive Quant C6 test result on Day 27.

Drug Administration: All treatments were administered orally once on Day 0.

Measurements and Observations: General health observations were conducted at least once daily. Blood samples were collected from each dog on Days -7, 27, 49, 63, 77, 91, and 104, and qualitatively tested for *B. burgdorferi* antibodies using the SNAP® 4Dx® Plus Test. Blood samples were also quantitatively assayed for *B. burgdorferi* antibodies using Lyme Quant C6® antibody tests. Four skin biopsies from each dog were collected on Day 104 from the heaviest areas of tick attachment, as marked on Day 33, and were tested by PCR for the quantitative presence of *B. burgdorferi*.

Statistical Methods*:*

Tick Counts: Percent effectiveness of the Simparica TRIO®-treated group with respect to the placebo-treated group was calculated for Day 33 using the formula [(C – T) / C] x 100, where C = arithmetic mean of live tick counts for the placebo-treated group and T = arithmetic mean of live tick counts for the Simparica TRIO®- treated group. Arithmetic means for live tick counts were estimated using the least squares means obtained from the statistical model. Tick counts were analyzed using a mixed linear model with treatment group as a fixed effect, and error as random effects. Treatment differences were assessed at the 5% level of significance.

Serology: A dog was considered to be infected with *B. burgdorferi* if a positive result was obtained on any of the SNAP® 4Dx® Plus tests for *B. burgdorferi* after Day 28, or the Lyme Quant C6® tests (titer ≥ 30 U/mL) after Day 28, or on PCR tests from any of the four skin biopsies collected on Day 104. For a dog to be considered not infected with *B. burgdorferi*, a negative result must be obtained for both serology tests at all sampling time points after Day 28 and for the PCR tests from all four skin biopsies collected on Day 104.

The proportion of animals infected (Yes/No) with *B. burgdorferi* in the Simparica TRIO®-treated group was compared to the proportion of animals infected in the placebo group using Fisher's Exact Test. The statistical test was performed at a significance level of 0.05 (two-sided).

Results: Placebo group dogs maintained adequate tick infestations on Day 33 with at least six of the nine dogs having 12 or more live ticks. The percent reduction in arithmetic mean live tick counts in the Simparica TRIO®-treated group compared to the placebo group on Day 33 was 100%. Mean live tick counts for the Simparica TRIO®-treated group was significantly lower than the placebo group ($P < 0.0001$).

Table II.2. *I. scapularis* Live Tick Effectiveness: Arithmetic Mean Live Tick Count (Percent Effectiveness)

Day of Tick Count	Placebo Group Arithmetic Mean Live Tick Count	Simparica TRIO® Arithmetic Mean Live Tick Count	Percent Effectiveness
33	13.4	0.0	100%

All dogs completing the study were seronegative for *B. burgdorferi* before treatment and tick infestations with negative test results on both the SNAP® 4Dx® Plus tests and Lyme Quant C6® tests (titer < 30 U/mL).

All nine placebo group dogs were determined to be infected with *B. burgdorferi* with positive test results obtained on the SNAP® 4Dx® Plus test for *B. burgdorferi* on and after Day 63, the Lyme Quant C6® test (titer ≥ 30 U/mL) on and after Day 49, and by the detection of *B. burgdorferi* on PCR from at least two of the four skin biopsies collected on Day 104.

Nine of ten Simparica TRIO®-treated dogs were determined to not have been infected with *B. burgdorferi* by remaining seronegative throughout the study with none of the SNAP® 4Dx® Plus tests for *B. burgdorferi* or the Lyme Quant C6® tests (titer ≥ 30 U/mL) positive at any timepoint, in addition to no detection of *B. burgdorferi* via PCR for all skin biopsies collected on Day 104, indicating prevention of *B. burgdorferi* infection. One Simparica TRIO®-treated dog had three positive Lyme Quant C6® tests (titer ≥ 30 U/mL) and a low positive detection of *B. burgdorferi* via PCR for one of the four skin biopsies collected. The proportion of dogs positive for *B. burgdorferi* in the Simparica TRIO®-treated group was significantly different than the placebo-treated group ($P = 0.0001$).

Table II.3. Serology and PCR Results for *B. burgdorferi:* SNAP® 4Dx® Plus Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO® -treated Dogs Positive for *B. burgdorferi*
-7	0/9	0/10
27	0/9	0/10
49	1/9	0/10
63	9/9	0/10
77	9/9	0/10
91	9/9	0/10
104	9/9	0/10

Table II.4. Serology and PCR Results for *B. burgdorferi:* Lyme Quant C6® Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO®-treated Dogs Positive for *B. burgdorferi*
-7	0/9	0/10
27	0/9	0/10
49	9/9	1/10
63	9/9	1/10
77	9/9	0/10
91	9/9	1/10
104	9/9	0/10

Table II.5. Serology and PCR Results for *B. burgdorferi:* PCR Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO®-treated Dogs Positive for *B. burgdorferi*
104	9/9	1/10

Adverse Reactions: There were no treatment-related adverse reactions during the study.

Conclusions: A single oral dose of Simparica TRIO® administered orally to dogs 28 days prior to infestation with *B. burgdorferi*-infected *I. scapularis* ticks prevented *B. burgdorfer*i infections as a direct result of killing the *I. scapularis* vector ticks.

2. Laboratory Dose Confirmation Study (Study No. A166C-US-20-B43)

Title: Evaluation of the Ability of Simparica TRIO® to Prevent the Transmission of *Borrelia burgdorferi* from Infected *Ixodes scapularis* to Dogs (Study A166C-US-20-B43)

Study Dates: November 09, 2020 to June 28, 2021

Study Location: Athens, GA

Study Design:

Objective: To evaluate the ability of Simparica TRIO® to protect dogs against *B. burgdorferi* infections from wild caught *I. scapularis* ticks by killing the ticks before transmission may occur.

Study Animals: Twenty (20) Beagle dogs (9 male and 11 female), 8 to 9 months of age, and 5.9 to 8.4 kg body weight.

Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with GCPs. The study included two treatment groups of 10 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel (as pamoate salt). The treatment was administered on Day 0. Each dog was infested with approximately 50 unfed, wild-caught, adult *I. scapularis* ticks (approximately equal numbers of males and females) on Day 28. The ticks had a *B. burgdorferi* infection rate of 75%. The ticks were counted and removed on Day 33.

Table II.6. Study Design for Study A166C-US-20-B43

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group	Day of Infestation	Day of Tick Count	Days of Blood Collection	Day of Skin Biopsy Collection
T01	Placebo	N/A	Day 0	10	28	33	-8, 27, 49, 63, 77, 91, and 104	104
T02	Simparica TRIO® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	10	28	33	-8, 27, 49, 63, 77, 91, and 104	104

[1] Minimum Dosage

Drug Administration: All treatments were administered orally once on Day 0.

Measurements and Observations:

General health observations were conducted at least once daily. Blood samples were collected from each dog on Days -8, 27, 49, 63, 77, 91, and 104, and qualitatively tested for *B. burgdorferi* antibodies using the SNAP® 4Dx® Plus Test. Blood samples were also quantitatively assayed for *B. burgdorferi* antibodies using Lyme Quant C6® antibody tests. Four skin biopsies from each dog were collected on Day 104 from the heaviest areas of tick attachment, as marked on Day 33, and were tested by PCR for the quantitative presence of *B. burgdorferi*.

Statistical Methods:

Tick Counts: Percent effectiveness of the Simparica TRIO®-treated group with respect to the placebo-treated group was calculated for Day 33 using the formula [(C – T) / C] x 100, where C = arithmetic mean of live tick counts for the placebo-treated group and T = arithmetic mean of live tick counts for the Simparica TRIO®- treated group. Arithmetic means for live tick counts were estimated using the least squares means obtained from the statistical model. Tick counts were analyzed using a mixed linear model with treatment group as a fixed effect and error as a random effect. Treatment differences were assessed at the 5% level of significance.

Serology: A dog was considered to be infected with *B. burgdorferi* if a positive result was obtained on any of the SNAP® 4Dx® Plus tests for *B. burgdorferi* after Day 28, or the Lyme Quant C6® tests (titer ≥30 U/mL) after Day 28, or on PCR tests from any of the four skin biopsies collected on Day 104. For a dog to be considered not infected with *B. burgdorferi*, a negative result must be obtained for both serology tests at all sampling time points after Day 28 and for the PCR tests from all four skin biopsies collected on Day 104.

The proportion of animals infected (Yes/No) with *B. burgdorferi* in the Simparica TRIO®-treated group was compared to the proportion of animals infected in the placebo group using Fisher's Exact Test. The statistical test was performed at a significance level of 0.05 (two-sided).

Results: Placebo dogs maintained adequate tick infestations on Day 33 with at least six of the ten dogs having 12 or more live ticks. The percent reduction in arithmetic mean live tick counts in the Simparica TRIO®-treated group compared to the placebo group on Day 33 was 100%. Mean live tick counts for the Simparica TRIO®-treated group was significantly lower than the placebo group ($P < 0.0001$).

Table II.7. *I. scapularis* Live Tick Effectiveness: Arithmetic Mean Live Tick Count (Percent Effectiveness)

Day of Tick Count	Placebo Group Arithmetic Mean Live Tick Count	Simparica TRIO® Arithmetic Mean Live Tick Count	Percent Effectiveness
33	23.4	0.0	100%

All dogs were seronegative for *B. burgdorferi* before treatment and tick infestations with negative test results on both the SNAP® 4Dx® Plus tests and Lyme Quant C6® tests (titer < 30 U/mL).

All ten placebo-treated dogs were determined to be infected with *B. burgdorferi* with positive test results obtained on the SNAP® 4Dx® Plus test for *B. burgdorferi* and the Lyme Quant C6® test (titer ≥ 30 U/mL) on and after Day 77, and the detection of *B. burgdorferi* on PCR from at least three of the four skin biopsies collected on Day 104.

All Simparica TRIO®-treated dogs were determined to not have been infected with *B. burgdorferi* by remaining seronegative throughout the study with none

of the SNAP® 4Dx® Plus tests for *B. burgdorferi* or the Lyme Quant C6® tests (titer ≥ 30 U/mL) positive at any timepoint, in addition there was no detection of *B. burgdorferi* via PCR for all skin biopsies collected on Day 104, indicating prevention of *B. burgdorferi* infection. One SNAP® 4Dx® test result on Day 91 for one dog was excluded due to an anomalous result. The proportion of dogs positive for *B. burgdorferi* in the Simparica TRIO®-treated group was significantly different than the placebo-treated group ($P < 0.0001$).

Table II.8. Serology and PCR Results for *B. burgdorferi:* SNAP® 4Dx® Plus Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO®-treated Dogs Positive for *B. burgdorferi*
-8	0/10	0/10
27	0/10	0/10
49	1/10	0/10
63	7/10	0/10
77	10/10	0/10
91	9/10	0/9*
104	10/10	0/10

* Only 9 dogs included at this time point due to an anomalous result for one dog.

Table II.9. Serology and PCR Results for *B. burgdorferi:* Lyme Quant C6® Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO®-treated Dogs Positive for *B. burgdorferi*
-8	0/10	0/10
27	0/10	0/10
49	6/10	0/10
63	10/10	0/10
77	10/10	0/10
91	10/10	0/10
104	10/10	0/10

Table II.10. Serology and PCR Results for *B. burgdorferi:* PCR Results

Study Day	Placebo-treated Dogs Positive for *B. burgdorferi*	Simparica TRIO®-treated Dogs Positive for *B. burgdorferi*
104	10/10	0/10

Adverse Reactions: No treatment-related adverse reactions were reported in this study.

Conclusions: A single oral dose of Simparica TRIO® administered orally to dogs 28 days prior to infestation with *B. burgdorferi*-infected *I. scapularis* ticks prevented *B. burgdorferi* infections as a direct result of killing the *I. scapularis* vector ticks.

For the treatment and control of L4 and immature adult Ancylostoma caninum

3. Laboratory Dose Confirmation Study (Study No. A166C-ZA-16-748: L4 *Ancylostoma caninum*)

   Title: Laboratory Dose Confirmation of Simparica TRIO® Against Induced Infections of Immature Stage (L4) of *Ancylostoma caninum* in Dogs

   Study Dates: April 12, 2017 to October 24, 2017

   Study Location: Bloemfontein, South Africa

   Study Design:

   Objective: Confirm the effectiveness of a single oral administration of Simparica TRIO® against induced infections of L4 *A. caninum* in dogs.

   Study Animals: Sixteen Beagle and mongrel/mixed-breed dogs (8 male and 8 female), 7-11 weeks of age, and 3.7 to 6.9 kg body weight.

   Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with GCPs. The study included two treatment groups of 8 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel (as pamoate salt). The treatment was administered on Day 0. Each dog was inoculated orally with 200 (±50) infective larvae (L3) of *A. caninum* 7 days prior to treatment administration.

Table II.11. Study Design for Study A166C-ZA-16-748

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group	Day of *A. caninum* Inoculation	Day of Necropsy and *A. caninum* Count Day of Tick Count
T01	Placebo	N/A	Day 0	8	-7	7
T02	Simparica TRIO® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	8	-7	7

[1] Minimum Dosage

Drug Administration: All treatments were administered orally once on Day 0.

Measurements and Observations: General health observations were conducted at least once daily. On Day 7 post-treatment all dogs were humanely euthanized and necropsied for recovery of *A. caninum*.

Statistical Methods:

Effectiveness was determined on the basis of the percentage reduction in *A. caninum* worm counts in the treated group compared to the control group.

For the log-transformed *A. caninum* worm counts, percent effectiveness of the treated group with respect to the placebo control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and block and error as random effects. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *A. caninum* infections. Effectiveness of Simparica TRIO® against L4 *A. caninum* is shown in Table II.12.

Table II.12. Effectiveness Against L4 *A. caninum* (Study A166C-ZA-16-748)

Treatment	***A. caninum* Worm Counts: Range**	***A. caninum* Worm Counts: Geometric Mean**	***A. caninum* Worm Counts: Percentage Reduction**
Placebo	4 to 17	9.2	NA
Simparica TRIO®	0 to 2	0.1[1]	98.4%

[1] The geometric mean worm count for the Simparica TRIO®-treated group was significantly lower than placebo ($P < 0.0001$)

Adverse Reactions: No treatment-related adverse reactions were reported in this study.

Conclusions: A single oral dose of Simparica TRIO® is effective in the treatment and control of immature Stage (L4) *A. caninum*.

4. Laboratory Dose Confirmation Study (Study No. A166C-ES-18-980: L4 *Ancylostoma caninum*)

Title: Laboratory Dose Confirmation of Simparica TRIO® Against Induced Infections of Immature Stage (L4) of *Ancylostoma caninum* in Dogs

Study Dates: November 18, 2018 to March 19, 2019

Study Location: Girona, Spain

Study Design:

Objective: Confirm the effectiveness of a single oral administration of Simparica TRIO® against induced infections of immature Stage (L4) of *A. caninum* in dogs.

Study Animals: Eighteen (18) Beagle and mixed-breed dogs (13 male and 5 female), 8 to 10 weeks of age, and 2.7 to 5.9 kg bodyweight.

Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with GCPs. The study included two treatment groups of 9 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel (as pamoate salt). The treatment was administered on Day 0. Each dog was inoculated orally with 200 (±50) infective larvae (L3) of *A. caninum* 7 days prior to treatment administration.

Table II.13. Study Design for Study A166C-ES-18-980

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group	Day of *A. caninum* Inoculation	Day of Necropsy and *A. caninum* Counts
T01	Placebo	N/A	Day 0	9	-7	7 and 8
T02	Simparica TRIO® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	9	-7	7 and 8

[1] Minimum Dosage

Drug Administration: All treatments were administered orally once on Day 0.

Measurements and Observations: General health observations were conducted at least once daily. On Day 7 or 8 post treatment all dogs were humanely euthanized and necropsied for recovery of *A. caninum*.

Statistical Methods:

Effectiveness was determined on the basis of the percentage reduction in *A. caninum* worm counts in the treated group compared to the control group.

For the log-transformed *A. caninum* worm counts, percent effectiveness of the treated group with respect to the placebo control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. A mixed linear

model analysis was used to analyze log-counts, with treatment group as a fixed effect, and room, block within room and error as random effects. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *A. caninum* infections.

Effectiveness of Simparica TRIO® against L4 *A. caninum* is shown in Table II.14.

Table II.14. Effectiveness Against L4 *A. caninum* (Study A166-ES-18-980)

Treatment	***A. caninum* Worm Counts: Range**	***A. caninum* Worm Counts: Geometric Mean**	***A. caninum* Worm Counts: Percentage Reduction**
Placebo	11 to 36	21	NA
Simparica TRIO®	0 to 0	0.0[1]	100%

[1] The geometric mean worm count for the Simparica TRIO®-treated group was significantly lower than placebo ($P < 0.0001$).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusions: A single oral dose of Simparica TRIO® is effective in the treatment and control of immature Stage (L4) *A. caninum*.

5. Laboratory Dose Confirmation Study (Study No. A166C-ZA-16-749: Immature Adult *Ancylostoma caninum*)

   Title: Laboratory Dose Confirmation of Simparica TRIO® Against Induced Infections of Immature Adult *Ancylostoma caninum* in Dogs

   Study Dates: July 20, 2017 to December 04, 2017

   Study Location: Bloemfontein, South Africa

   Study Design:

   Objective: Confirm the effectiveness of a single oral administration of Simparica TRIO® against induced infections of immature adult *A. caninum* in dogs.

   Study Animals: Sixteen (16) Beagle and mixed-breed dogs (8 male and 8 female), 7 to 11 weeks of age, and 4.0 to 9.0 kg bodyweight.

   Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with GCPs. The study included two treatment groups of 8 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel

(as pamoate salt). The treatment was administered on Day 0. Each dog was inoculated orally with 200 (±50) infective larvae (L3) of *A. caninum* 11 days prior to treatment administration.

Table II.15. Study Design for A166C-ZA-16-749

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group	Day of *A. caninum* Inoculation	Day of Necropsy and *A. caninum* Counts
T01	Placebo	N/A	Day 0	8	-11	7
T02	Simparica TRIO ® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	8	-11	7

[1] Minimum Dosage

Drug Administration: All treatments were administered orally once on Day 0.

Measurements and Observations: General health observations were conducted at least once daily. On Day 7 post-treatment all dogs were humanely euthanized and necropsied for recovery of *A. caninum*.

Statistical Methods:

Effectiveness was determined on the basis of the percentage reduction in *Ancylostoma caninum* worm counts in the treated group compared to the control group.

For the log-transformed *A. caninum* worm counts, percent effectiveness of the treated group with respect to the placebo control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect and block and error as random effects. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *A. caninum* infections.

Effectiveness of Simparica TRIO® against Immature Adult *A. caninum* is shown in Table II.16.

Table II.16. Effectiveness Against Immature Adult *A. caninum* (Study A166C-ZA-16-749)

Treatment	*A. caninum* Worm Counts: Range	*A. caninum* Worm Counts: Geometric Mean	*A. caninum* Worm Counts: Percentage Reduction
Placebo	110 to 260	209.9	NA
Simparica TRIO®	0 to 3	0.5[1]	99.8%

[1] The geometric mean worm counts for the Simparica TRIO® -treated group was significantly lower than placebo ($P < 0.0001$).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusions: A single oral dose of Simparica TRIO® is effective in the treatment and control of immature adult *A. caninum*.

6. Laboratory Dose Confirmation Study (Study No. A166C-ZA-18-959: Immature Adult *Ancylostoma caninum*)

Title: Laboratory Dose Confirmation of Simparica TRIO® Against Induced Infections of Immature Adult *Ancylostoma caninum* in Dogs

Study Dates: January 11, 2019 to March 20, 2019

Study Location: Bloemfontein, South Africa

Study Design:

Objective: Confirm the effectiveness of a single oral administration of Simparica TRIO® against induced infections of immature adult *A. caninum* in dogs.

Study Animals: Sixteen (16) Beagle and mixed-breed dogs (8 male and 8 female), 9 to 10 weeks of age, and 4.6 to 7.0 kg bodyweight.

Experimental Design: This study was a placebo-controlled, masked, completely randomized study design conducted in accordance with GCPs. The study included two treatment groups of 8 dogs per group. One group was administered a placebo and one group was administered Simparica TRIO® at a dose of 1.2 mg/kg sarolaner, 24 µg/kg moxidectin, and 5 mg/kg pyrantel (as pamoate salt). The treatment was administered on Day 0. Each dog was inoculated orally with 200 (±50) infective larvae (L3) of *A. caninum* 11 days prior to treatment administration.

Table II.17. Study Design for Study A166C-ZA-18-959

Treatment Group	Treatment	Dosage[1]	Day of Treatment	Dogs per Group	Day of *A. caninum* Inoculation	Day of Necropsy and *A. caninum* Counts
T01	Placebo	N/A	Day 0	8	-11	7
T02	Simparica TRIO® (sarolaner + moxidectin + pyrantel (as pamoate salt))	1.2 mg/kg + 24 µg/kg + 5 mg/kg	Day 0	8	-11	7

[1] Minimum Dosage

Drug Administration: All treatments were administered orally.

Measurements and Observations: General health observations were conducted at least once daily. On Day 7 post-treatment all dogs were humanely euthanized and necropsied for recovery of *A. caninum*.

Statistical Methods:

Effectiveness was determined on the basis of the percentage reduction in *A. caninum* worm counts in the treated group compared to the control group.

For the log-transformed *A. caninum* worm counts, percent effectiveness of the treated group with respect to the placebo control group was calculated using the formula [(C-T)/C] x 100, where C = geometric mean (back-transformed mean) of worm counts for the control group and T = geometric mean (back-transformed mean) of worm counts for the treated group. A mixed linear model analysis was used to analyze log-counts, with treatment group as a fixed effect, and room, block within room and error as random effects. Treatment differences were assessed at the 5% level of significance.

Results: Control dogs had adequate *A. caninum* infections.

Effectiveness of Simparica TRIO® against immature adult *A. caninum* is shown in Table II.18.

Table II.18. Effectiveness Against Immature Adult *A. caninum* (Study A166C-ZA-18-959)

Treatment	*A. caninum* Worm Counts: Range	*A. caninum* Worm Counts: Geometric Mean	*A. caninum* Worm Counts: Percentage Reduction
Placebo	23 to 41	29.4	NA
Simparica TRIO®	0 to 0	0.0[1]	100%

[1] The geometric mean worm count for the Simparica TRIO®-treated group was significantly lower than placebo (P <0.0001).

Adverse Reactions: No adverse reactions related to treatment were reported in this study.

Conclusions: A single oral dose of Simparica TRIO® is effective in the treatment and control of immature adult *A. caninum*.

III. TARGET ANIMAL SAFETY

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-521, dated February 27, 2020, contains a summary of target animal safety studies for dogs.

IV. HUMAN FOOD SAFETY

This drug is intended for use in dogs. Because this new animal drug is not intended for use in food-producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to Simparica TRIO®.

Not for use in humans. Keep this and all drugs out of the reach of children.

Keep Simparica TRIO® in a secure location out of reach of dogs, cats and other animals to prevent accidental ingestion or overdose.

VI. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act (FD&C Act) and 21 CFR part 514. The data demonstrate that Simparica TRIO®, when used according to the label, is safe and effective for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks and for the treatment and control of L4 and immature adult *Ancylostoma caninum*.

A. Marketing Status

The drug is restricted to use by or on the order of a licensed veterinarian. Adequate directions for lay use cannot be written because the product is indicated for the prevention of heartworm infections *(D. immitis)* in dogs, which requires veterinary examination and testing to ensure dogs are negative for adult heartworm disease prior to administration of the product to dogs.

B. Exclusivity

This supplemental approval for Simparica TRIO® qualifies for THREE years of marketing exclusivity under section 512(c)(2)(F)(iii) of the FD&C Act because the supplemental application included effectiveness studies. This exclusivity begins as of the date of our approval letter and only applies to the indications, "for the prevention of *Borrelia burgdorferi* infections as a direct result of killing *Ixodes scapularis* vector ticks" and "for the treatment and control of L4 and immature adult *Ancylostoma caninum*."

C. Supplemental Applications

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.