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Determination of the effective dose of a novel oral formulation of sarolaner (Simparica™) for the treatment and month-long control of fleas and ticks on dogs

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ABSTRACT

Three laboratory studies were conducted to determine the appropriate dose of sarolaner, a novel isoxazoline, for the treatment and month-long control of infestations of fleas and ticks on dogs. In the first study, dogs were treated orally with sarolaner suspension formulations at 1.25, 2.5 or 5.0 mg/kg, and infested with *Dermacentor reticulatus, Rhipicephalus sanguineus* ticks and with *Ctenocephalides felis felis* (cat flea) prior to treatment and then weekly for up to 8 weeks. Fleas and ticks were counted 48 h after treatment and after each subsequent infestation at 24 h for fleas and 48 h for ticks. The lowest dose of sarolaner (1.25 mg/kg) provided 100% efficacy against fleas from treatment through Day 35 and 98.4% at Day 56. This dose of sarolaner resulted in 99.7–100% control of both species of ticks through Day 28. In Study 2, dogs were dosed orally with placebo or sarolaner suspension formulations at 0.625, 1.25 or 2.5 mg/kg and infested with *Ixodes scapularis* prior to treatment and weekly for 6 weeks, *Amblyomma americanum* (pretreatment and Day 26), *Dermacentor variabilis* (Day 33) and *A. maculatum* (Day 41). *Ixodes scapularis* was the most susceptible; the lowest dose (0.625 mg/kg) providing >95% efficacy through Day 43. Efficacy against *D. variabilis* on Day 35 was >95% at 1.25 and 2.5 mg/kg, whereas the 0.625 mg/kg dose gave only 61.4% efficacy. *Amblyomma* spp. were the least susceptible ticks; efficacy of the 1.25 mg/kg dose at Day 28 for *A. americanum* was markedly lower (88.5%) than achieved for *D. reticulatus* (100%) at Day 28 and also lower than for *D. variabilis* at Day 35 (96.2%). In Study 3, dogs were dosed orally with placebo or sarolaner in the proposed commercial tablet (Simparica™) at 1.0, 2.0 or 4.0 mg/kg, and infested with *A. maculatum*, one of the ticks determined to be dose limiting, prior to treatment and then weekly for 5 weeks. All doses gave 100% control of the existing infestation. The two highest dosages resulted in >93% control of subsequent challenges for 5 weeks. There was no significant improvement in efficacy provided by the 4.0 mg/kg dose over the 2.0 mg/kg dose ($P > 0.05$) at any time point. The 2.0 mg/kg dose was superior to the 1.0 mg/kg on Day 14 ($P = 0.0086$) and as efficacy for 1.0 mg/kg declined below 90% at Day 28, a single 1 mg/kg dose would not provide a full month of tick control. Thus, 2.0 mg/kg was selected as the sarolaner dose rate to provide flea and tick control for at least one month following a single oral treatment.

1. Introduction

The most common permanent or semi-permanent ectoparasites occurring on dogs are fleas, ticks and mites. *Ctenocephalides felis felis,* the cat flea, is considered the most important ectoparasite of dogs and cats (Rust and Dryden, 1997) and is endemic worldwide. Adult fleas are blood feeders, are recognized as a major cause of allergic skin disease in dogs, and when present in sufficient numbers are capable of causing anemia (Krämer and Mencke, 2001). They are intermediate hosts for the dog tapeworm, *Dipylidium caninum,* and can transmit a number of zoonotic pathogens, including *Bartonella henselae*, the causative agent for cat scratch fever, as well as other zoonotic Bartonellas, such as *B. clarrigeae* and *B. koehlerae* (Chomel and Kasten, 2010).

Tick infestations can range from an occasional nuisance to a continuous infestation and can cause serious, even life-threatening disease (Dryden and Payne, 2004). During feeding, large amounts of blood are taken up by ticks; excess water is removed and

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returned to the host in the form of saliva, which contains a number of pharmacologically active substances, including anticoagulants and immunomodulators, with the components varying between species. Tick-borne pathogens are normally passed to their next host in saliva and some species excrete toxins within their saliva (Needham and Teel, 1991). In severe infestations, tick numbers may be high enough to cause anemia due to physical blood loss through their feeding. Ticks are responsible for the transmission of a number of pathogens, some dog-specific, and others zoonotic. *Babesia canis* and *Ehrlichia canis* are both primarily dog-specific infections, the former primarily transmitted by *Dermacentor* spp. (*D. variabilis*, American Dog tick and *D. reticulatus*, Ornate Cow tick) and the latter by the Brown Dog tick, *Rhipicephalus sanguineus* (Chomel, 2011; Dantas-Torres et al., 2012). Zoonotic infections include Lyme disease, caused by *Borrelia burgdorferi,* and human granulocytic anaplasmosis (HGA), caused by *Anaplasma phagocytophilum,* which are transmitted by *Ixodes* spp., and Rocky Mountain Spotted Fever, caused by *Rickettsia rickettsii,* which is transmitted primarily by ticks in the genera *Amblyomma, Dermacentor, Ixodes* (Dryden and Payne, 2004; Chomel, 2011) and *Rhipicephalus* (Demma et al., 2005). *Amblyomma americanum,* the Lone Star tick, and *A. maculatum*, the Gulf Coast tick, have been determined to harbor a number of other *Erlichia, Borrelia* and *Rickettsia* spp. (Mixon et al., 2006; Jiang et al., 2012; Moncayo et al., 2010; Nicholson et al., 2009; Paddock et al., 2010).

Control of fleas and ticks is primarily based on the use of parasiticides, and until recently convenient on-animal treatments applied as spot-on or collar applications have been the standard accepted method (Dryden and Payne, 2004; Rust, 2005). The most widely used products include compounds with efficacy against fleas and ticks, as well as specific insecticides or acaricides. Most have direct insecticidal/acaricidal activity and control the parasites on the animal. In addition, there are products such as insect growth regulators that may be orally dosed or applied to the pet and control fleas by disrupting the off-host life stages (eggs and larvae), and others that may be used for environmental applications. Despite the variety of available products and application methods, both fleas and ticks remain an ongoing problem for many pet owners. Formulations of spinosad (for fleas only) and more recently, isoxazoline insecticide/acaricides have been introduced that provide control of these ectoparasites for a month or more after a single oral dose (Robertson-Plouch et al., 2008; Rohdich et al., 2014; Shoop et al., 2014). There are obvious advantages to orally administered products, which remove environmental/user exposure concerns that may accompany topically applied products. In addition, oral products are not affected by bathing/water exposure as are some topically administered products, thus ensuring a more uniform performance during the treatment period.

Sarolaner is a novel isoxazoline with potent activity against fleas and ticks that was developed specifically for companion animals (McTier et al., 2016). Here we report studies conducted to determine the appropriate dose rate of an oral formulation of sarolaner (Simparica™, Zoetis) given as a single dose to dogs to provide treatment of existing infestations and at least one month control of fleas and ticks.

2. Materials and methods

Three studies were conducted to: 1) compare the relative susceptibilities of fleas and ticks; 2) determine which of the common ticks found on dogs in the US was the least susceptible and; 3) identify the minimum dose required to treat and control the least sensitive parasite (s) for at least one month following a single treatment.

Study 1 was conducted in South Africa and Studies 2 and 3 were conducted in the USA. The studies were conducted in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestation on dogs and cats (Marchiondo et al., 2013). Study 3 additionally complied with Good Clinical Practices, (VICH guideline GL9) (EMEA, 2000). Study protocols were reviewed and approved by the ClinVet and/or Zoetis Institutional Animal Care and Use Committee (s). Masking of all studies was assured through the separation of functions. All personnel conducting observations or animal care, or performing infestations and counts were masked to treatment allocation.

2.1. Animals

Individually identified, adult, purpose-bred Beagles ≥8 months of age and ≤ 20 kg were used in each study. The dogs had not been treated with an ectoparasiticide for at least 60 days and were in good health at the time of treatment. Dogs were housed individually in indoor runs that conformed to accepted animal welfare guidelines. Dogs were fed an appropriate maintenance ration of a commercial dry canine feed for the duration of the study. Water was available *ad libitum*. Study 1 included 48 dogs (24 male and 24 female), Study 2 used 32 male dogs and Study 3 had 32 dogs (15 male, 17 female).

2.2. Experimental design and methods

General methods: Day 0 represents the day that dogs received the study treatment. Dogs were acclimated to the study conditions for at least 14 days prior to treatment. The dogs were observed for general health at least once daily throughout the studies. A physical exam was performed on each dog by a veterinarian to determine health and suitability prior to inclusion in the study. For infestations in Studies 1 and 2, approximately 100 cat fleas (*C. felis felis*, ~1:1 sex ratio) and/or approximately 50 ticks (~1:1 sex ratio) of each species to be assessed were applied directly to a site proximal or adjacent to the shoulder blades and allowed to crawl into the hair coat. In study 3, dogs were sedated prior to tick infestation and confined in shipping crates for approximately 6 h to reduce animal movement and enhance tick attachment. Forty eight hours after treatment and each tick infestation and/or 24 h after each flea infestation, every dog was thoroughly examined and combed to remove and count fleas and ticks. Flea and tick counts were performed by personnel trained in the standard procedures in use at the test facility. Protective gloves and clothing were changed between dogs, and personnel conducting parasite counts or other observations were unaware of treatment assignments.

Study 1: On Day−7, each dog was infested with brown dog ticks (*R. sanguineus*). On Day−5, the ticks on each dog were removed and counted; dogs were ranked by descending tick count into eight blocks of six, and randomly allocated within blocks to six treatment groups. On Day−2, the dogs were weighed and infested with *R. sanguineus* and *Dermacentor reticulatus* ticks. On Day−1, the dogs were infested with fleas.

On Day 0, the eight animals in each group were treated via oral gavage at 0.5 mL/kg body weight with one of the following: placebo; sarolaner suspension (10 mg/mL) to provide a dose of 5 mg/kg; sarolaner suspension (5 mg/mL) to provide a dose of 2.5 mg/kg; sarolaner suspension (2.5 mg/mL) to provide a dose of 1.25 mg/kg; another isoxazoline analog suspension (5 mg/mL) to provide a dose of 2.5 mg/kg (results not provided) or sarolaner solution (5 mg/mL) to provide a dose of 2.5 mg/kg. The results for the latter experimental treatment are reported in McTier et al. (2016). Dogs were observed for general health and any reaction to treatment approx-