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The prevention of attachment and the detachment effects of a novel combination of fipronil, amitraz and (S)-methoprene for *Rhipicephalus sanguineus* and *Dermacentor variabilis* on dogs

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ABSTRACT

A novel combination of fipronil, amitraz and (S)-methoprene (CERTIFECT™, Merial Limited, GA, USA) was evaluated for the prevention of attachment of ticks and its ability to cause detachment of ticks. For the two prevention of attachment studies, 20 purpose-bred beagles were allocated each to two equal groups based on pretreatment tick counts (treated and untreated). Each dog was exposed to 50 adult *Rhipicephalus sanguineus* and *Dermacentor variabilis* weekly starting 24 h after treatment. In study 1 infestations with *R. sanguineus* were discontinued after Day 7 but continued to Day 28 for *D. variabilis* in both studies. Counts of ticks by species were made 2, 4 and 24 h after exposure to ticks. Ticks not attaching to dogs were evaluated for viability. For the evaluation of detachment study, 16 purpose-bred beagles were allocated each to two equal groups based on pretreatment tick counts (treated and untreated). Each dog was infested with 50 unfed *R. sanguineus* and *D. variabilis* adults on Day -2. Ticks were thumb counted without removal on all dogs on Day -1, and at 4, 12, and 24 h after treatment. Ticks were counted and removed at 48 h after treatment.

Dogs treated with the novel combination had significantly ($p < 0.05$) lower total numbers of attached *R. sanguineus* and *D. variabilis* than untreated controls at 4 h through Day 7. For *R. sanguineus*, percent reduction of attachment at 24 h after infestation through Day 29 ranged from 94.5% to 100%. For *D. variabilis*, the percent reduction of attachment at 24 h through Day 22 was above 98.0%. These studies demonstrate that novel combination can disrupt attachment of *R. sanguineus* and *D. variabilis* for up to 28 days following treatment. Of those ticks that are exposed to the treatment, even if they do not attach to the dog and remain in the environment, greater than 90% ($p < 0.05$) die within 24 h for 2–3 weeks following treatment. Also, for those dogs infested with ticks at the time of treatment, the novel combination causes significant detachment ($p < .05$) starting at 12 h and reaching 98.9% by 48 h after treatment. This product provides an effective means for controlling ticks infesting dogs and limiting the spread of tick transmitted diseases. Additionally, the mortality of ticks exposed to CERTIFECT will reduce infestation of the dog's environment.

1. Introduction

Rhipicephalus sanguineus, the brown dog tick, is one of the most widely distributed tick species, occurring world-

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wide primarily between the latitudes of 35°S and 50°N. It shows a strong preference for feeding on dogs and having dogs as a host may be a necessary condition for developing large populations (Dantas-Torres, 2008). This characteristic, in combination with its ability to infest and thrive in home and kennel environments, makes it a common and troublesome parasite of dogs. Although not as specific to dogs as *R. sanguineus, Dermacentor variabilis*, the American dog tick, is also a common parasite of dogs and occurs primarily over the eastern half of the United States (Dryden and Payne, 2004). It can be found from southern New England to Florida and from the east coast to the plains states and in Canada east of Saskatchewan. Populations of the tick are also found on the Pacific coast.

Rhipicephalus sanguineus and *D. variabilis* can cause direct harm through irritation produced during attachment and feeding on the host and by causing anemia when found in sufficient numbers (Bowman, 2008; Urquhart et al., 2003). *Rhipicephalus sanguineus* is a known vector of *Ehrlichia canis* and *Babesia canis canis*, two blood borne pathogens that can cause life threatening alterations in red blood cell homeostasis (Shaw et al., 2001). *Dermacentor variabilis* is a known vector of *Rickettsia rickettsii* and *Francisella tularensis* and is a direct cause of tick paralysis (Dryden and Payne, 2004). Providing protection to the dog from these disease vectors is an important component of maintaining their health.

Effective and rapid control of ticks is important to reduce irritation produced by ticks and to reduce the chance of transmission of pathogens to dogs and potentially to their owners (Jacobson et al., 2004; Davoust et al., 2003). On-animal treatments in spot-on formulations provide convenience in ease of use and a monthly dosing interval. The currently available active components, fipronil, amitraz, and permethrin, have the greatest activity against ticks (Dryden and Payne, 2004). In addition fipronil and permethrin have been shown to prevent transmission of disease to dogs (Davoust et al., 2003; Jacobson et al., 2004; Otranto et al., 2008).

Previous studies have investigated the repellent and detachment effects of fipronil and amitraz separately but not in combination (Elfassy et al., 2001; Young et al., 2004; Dryden et al., 2006). The purpose of the studies reported here were to investigate the ability of the combination of fipronil, amitraz and (S)-methoprene (CERTIFECT™, Merial Limited, GA, USA) to prevent attachment of ticks and to cause detachment of ticks that have already attached. Ticks that had been exposed to treated dogs but that had not attached were assessed at 24 h for viability.

2. Materials and methods

2.1. Animals

All studies were conducted according to the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products Guideline 9: Good Clinical Practice and in compliance with local animal welfare legislation and were approved by an Independent Animal Care and Use Committee (Anonymous, 2000).

In Study 1 twenty-one purpose bred beagle dogs, 12 males and 9 females (>6 months old; 9.65–16.10 kg), were acclimatised to the test facility for at least 7 days prior to treatment (Study Day 0). In Study 2 twenty-four purpose bred beagle dogs, 13 males and 11 females (>6 months old; 8.05–17.10 kg), were acclimatised to the test facility for 7 days prior to treatment. In the detachment study (Study 3) nineteen beagle dogs, 10 males and 9 females, (>6 months old; 6.8–17.0 kg) were acclimatised to the test facility for 14 days prior to treatment. For each study every dog was uniquely identified with an ear tattoo. Dogs used in these studies had no exposure to topical or systemic ectoparasiticides for at least 3 months prior to the start of the studies. Dogs were washed with a non-insecticidal shampoo on Day -13 in Study 1, Day -7 in Study 2 and Day -14 in the detachment study.

Dogs were housed individually in indoor cages or runs that prevented contact between dogs from different treatment groups. Each cage or run was uniquely identified with the dog's identification and was not identified by treatment. All dogs were fed a commercial diet and water was available *ad libitum*.

Personnel wore protective gloves and gowns that were changed between treatment groups to prevent cross-contamination. Separate tables were assigned to each treatment group or the exam table was cleaned with alcohol and wiped dry between treatment groups. Separate forceps and flea combs were used for tick collections for each treatment group in all studies.

2.2. Experimental design—prevention of attachment

Each dog was infested with 50 unfed *R. sanguineus* adult ticks on Day -6 for Study 1 and Day -5 for Study 2 by placing ticks in a crate and then placing the dog in the crate. The dogs were kept in individual exposure crates for 2 h and then returned to their cages or runs. The ticks were counted and removed from the dogs at 48 h after infestation for allocation purposes only. The dogs were ranked in descending order by tick count and randomly allocated to one of two treatment groups in each study. One dog in Study 1 and four dogs in Study 2 with the lowest tick counts were not allocated to treatment. This resulted in two treatment groups of 10 animals each for each study. Dogs were weighed on Day -4 for Study 1 and Day -3 for Study 2. The control group was untreated (Treatment Group 1). Treatment Group 2 had the novel combination based on 2 formulations applied concurrently on Day 0. The formulations consisted of one containing 10% (w/v) fipronil and 9% (w/v) (S)-methoprene and a second containing 20% (w/v) amitraz. Dogs weighing 10.0 kg or less were treated with 0.67 mL of the fipronil and (S)-methoprene formulation and 0.40 mL of the amitraz formulation. Dogs weighing more than 10 kg and up to 20 kg were treated with 1.34 mL of the fipronil and (S)-methoprene formulation and 0.80 mL of the amitraz formulation. This resulted in dose rates of at least 6.7 mg fipronil/kg body weight (bw), 8.0 mg amitraz/kg bw and 6.0 mg (S)-methoprene/kg bw. Treatment was applied by parting the hair and concurrently applying the two formulations from 2 syringes directly onto the skin, divided in two approximately equal volumes, each applied