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Efficacy of a novel topical combination of fipronil, amitraz and (S)-methoprene for treatment and control of induced infestations with four North American tick species (*Dermacentor variabilis*, *Ixodes scapularis*, *Amblyomma americanum* and *Amblyomma maculatum*) on dogs

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ABSTRACT

Five laboratory studies were conducted to confirm that a single topical dose of the novel combination of fipronil, amitraz and (S)-methoprene, CERTIFECT™ (Merial Limited, GA, USA), is efficacious for the rapid control of pre-existing infestations and the prevention of new infestations with *Ixodes scapularis*, *Dermacentor variabilis*, *Amblyomma americanum* and *Amblyomma maculatum* for at least 28 days on dogs. In each study, 8 male and 8 female purpose-bred, laboratory beagles were randomly assigned to one of two study groups (treated and untreated). Starting on the day before treatment, each dog was infested weekly with about 30 or 50 ticks, depending on the study. Treatment with the novel combination rapidly eliminated pre-existing infestations and controlled weekly re-infestations for at least 28 days. Pre-existing infestations with all four tick species were rapidly and effectively reduced, with post-treatment therapeutic efficacies ranging from 91.7 to 99.5% within 18–48 h post treatment. *Amblyomma maculatum* numbers were significantly ($p < 0.05$) reduced on treated dogs from the first tick counts as early as 6 h post-treatment. All subsequent infestations with each of the 4 tick species were quickly disrupted, with prophylactic efficacies greater than 90% within 18–48 h post-infestation for at least a full month. Because the combination of fipronil, amitraz and (S)-methoprene quickly starts disrupting and killing ixodid ticks within hours of treatment, with similar high levels of efficacy maintained for at least 28 days in these and other studies, the authors conclude that a single topical treatment with CERTIFECT may prevent the transmission of most infectious agents carried by ixodid ticks for at least one month.

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1. Introduction

Tick control is an important animal and public health concern. Heavy and prolonged tick infestations can cause anemia, especially in young or small dogs. Several tick species may carry infectious agents or toxins, which they may transmit to their host if they remain attached for sufficient time. These tick-borne infectious agents may cause sub-clinical to life-threatening infections in dogs and human beings (Blagburn and Dryden, 2009; Nicholson et al., 2010). Several North American tick species also may transmit a salivary neurotoxin that can block the pre-synaptic release of acetylcholine at neuromuscular junctions and cause an acute ascending flaccid paralysis in dogs and humans (Nafe, 1988; Keirans et al., 1996; Braund, 2003).

The American dog tick, *Dermacentor variabilis* (also referred to as wood tick), is widely found in North and South America. It is the principal vector of *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever in dogs and humans (CDC, 2009). It also can transmit *Ehrlichia canis* (agent of canine ehrlichiosis), *Francisella tularensis* (agent of tularemia), *Ehrlichia ewingii* or *E. chaffeensis* (agents of ehrlichiosis in people and dogs), and *Babesia canis canis*, which causes babesiosis (Johnson et al., 1998; Steiert and Gilfoy, 2002; Yabsley et al., 2008). North American *D. variabilis* and other *Dermacentor* species also can cause tick paralysis (Blagburn and Dryden, 2009).

Ixodes spp. ticks, including *I. scapularis* (blacklegged or deer ticks), are widely distributed in North America, where they are the main vectors of *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, the agents of Lyme borreliosis and granulocytic anaplasmosis in dogs and humans. They also may carry babesiosis and ehrlichiosis agents, *e.g. Babesia microti* and *Ehrlichia* spp. (Varde et al., 1998). *Ixodes scapularis* also may cause tick paralysis (Blagburn and Dryden, 2009).

Amblyomma americanum (Lone Star tick) is mainly found in wooded areas from Florida and Texas to Iowa, extending to the East Coast and up to Maine (Blagburn and Dryden, 2009). It is the main vector for *E. ewingii* and *E. chaffeensis* (agents of ehrlichiosis in people). It also can carry STARI (unidentified agent of Southern Tick-Associated Rash Illness), *F. tularensis* (agent of tularemia), and Panola Mountain *Ehrlichia* species (Steiert and Gilfoy, 2002; Reeves et al., 2008; Goddard and Varela-Stokes, 2009).

Amblyomma maculatum (Gulf Coast tick) is endemic in the United States in areas bordering the Gulf of Mexico, in Alabama, Arizona, Arkansas, Florida, Georgia, Indiana, Kansas, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, Virginia, West Virginia, and in countries of Central and South America around and beyond the Gulf of Mexico and the Caribbean Sea, including Mexico, Guatemala, Belize, Nicaragua, Honduras, Costa Rica, Colombia, Venezuela, and parts of Ecuador and Peru (Sumner et al., 2007; Kasari et al., 2010). *A. maculatum* is the proven vector of *Hepatozoon americanum* and may be involved in transmission of spotted fever rickettsial agents, and tick paralysis toxin (Blagburn and Dryden, 2009; Holman and Snowden, 2009).

Prevention of tick infestations and rapid disruption of tick feeding are the best protection against the transmission of infectious agents and toxins carried by ticks. This paper summarizes five studies conducted to confirm the efficacy of a single topical application of a novel combination of fipronil, amitraz and (S)-methoprene (CERTIFECT™, Merial Limited, GA, USA) against induced infestations with four North American ixodid tick species: *D. variabilis, I. scapularis*, *A. americanum* and *A. maculatum*. The efficacy of CERTIFECT against *Rhipicephalus sanguineus* (brown dog tick) infestations is described by Hunter et al. (2011) elsewhere in this volume.

2. Materials and methods

2.1. Experimental design

Five studies were conducted to confirm that the combination of fipronil, amitraz, and (S)-methoprene is efficacious against North American tick species, including *D. variabilis, I. scapularis, A. americanum* and *A. maculatum* (2 studies). All studies followed published methods (Marchiondo et al., 2007), Good Clinical Practice Guideline 9 of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products, and the Guidelines for the testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cats, EMEA/CVMP/EWP/005/2000 (The European Medicines Agency, Committee for Medicinal Products for Veterinary Use, London, 12 November 2007).

Each study utilized unfed adult ticks from laboratory-maintained populations that had been established from ticks collected in field locations in the USA.

All dogs were healthy, purpose-bred laboratory beagles (7.6 months to 11 years old). They had not been treated with an ectoparasiticide within three months prior to study initiation and were managed with due regard for their well being, in accordance with local Institutional Animal Care and Use Committee requirements and international laws and ethics, including the International Guiding Principles for Biomedical Research Involving Animals issued by the Council for the International Organizations of Medical Sciences. All studies followed the same controlled, blinded, and randomized design. The main activities in each study are listed in Table 1. A veterinary examination showed that all dogs were healthy and free of abnormalities at the treatment site. The dogs were individually penned and observed daily for any health changes. A pre-treatment tick infestation was conducted on 20 dogs in each study to assess their susceptibility to infestations, and eight replicates of two dogs were formed based on descending pre-treatment tick counts within sex for each study. Within replicates, 8 male and 8 female dogs were randomly allocated to one of the two treatment groups, and the 2 males and 2 females with lowest tick counts were excluded from the study.

2.2. Methods

Dogs in group 1 were not treated (untreated, control group), and those in group 2 were treated once topically