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Field evaluation of the efficacy and safety of a combination of spinosad and milbemycin oxime in the treatment and prevention of naturally acquired flea infestations and treatment of intestinal nematode infections in dogs in Europe

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ABSTRACT

Two separate randomised, blinded, multicentre field trials were conducted to evaluate the efficacy and safety of a combination of spinosad and milbemycin oxime (MO) (Trifexis®, Elanco Animal Health) in the treatment and prevention of naturally acquired flea infestations and intestinal nematode infections in European dogs. Treatments using Trifexis® and each control veterinary product (CVP) were administered once on Day 0 in both field studies.

In the flea field trial, 11 veterinary clinics in France participated in the study. On Day 0, whole body flea comb counts were conducted on all dogs being evaluated for enrolment. Dogs with ≥7 fleas on Day 0 were enrolled, treated once on Day 0 with spinosad/MO or the CVP (Stronghold®; selamectin) and then underwent post-treatment flea counts on Days 14 and 30. There were 150 spinosad/MO treated dogs and 71 CVP treated dogs included in the flea effectiveness population. Effectiveness against fleas (% reduction in geometric means; GM) was 98.97% and 97.37% for the spinosad/MO treated dogs, and 97.43% and 93.96% for the CVP dogs on Days 14 and 30, respectively, compared to the pre-treatment baseline flea counts. Of the spinosad/MO dogs, 89.3% and 80.0% had no live fleas on Days 14 and 30, compared to 77.5% and 70.4% of the CVP dogs, respectively.

In the nematode field trial, data from 10 veterinary clinics in France and 19 in Ireland were pooled. Faecal samples from dogs at each clinic were analysed. A positive result at screening (parasite eggs from *Toxocara canis*, *Toxascaris leonina*, *Trichuris vulpis* or *Ancylostoma caninum*) allowed for enrolment. Dogs were randomised to spinosad/MO or the CVP (Milbemax®; MO/praziquantel). On Day 8, a post-treatment faecal sample was taken and analysed. Of 2333 dogs screened for nematode eggs, 238 dogs were positive with one or more of these nematodes, and 229 were enrolled in the study. Of the 229 dogs, 151 were treated with a single dose of spinosad/MO, and 77 were treated with a single dose of CVP. Post-treatment effectiveness against all nematodes (% reduction GM) was achieved with reductions of 98.57% and 97.57% for the spinosad/MO treated dogs and CVP dogs, respectively, as compared to the pre-treatment baseline faecal egg counts.

Trifexis® was shown to be safe and effective against natural infestations of fleas as well as mixed and single intestinal nematode infections in client owned dogs in Europe when administered as a single oral administration at the recommended dose.

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1. Introduction

The dog and cat flea species (*Ctenocephalides canis* and *Ctenocephalides felis*) are considered the most important insect ectoparasites of companion animals worldwide and may readily infest humans (Halos et al., 2014). Several studies have highlighted the high rate of flea infestations that are common in companion animals, varying between 12% and 47% in some European countries (Halos et al., 2014) and most of these documented infestations are with the predominant flea species of dogs and cats, *C. felis*. Infections with intestinal nematodes are also common in dogs from all parts of the world, including Europe (Grandemange et al., 2007; Little et al., 2009; Neves et al., 2014; Riggio et al., 2013). The nematodes, *Ancylostoma caninum* and *Toxocara canis*, are considered two of the most important intestinal helminth parasites of dogs with infections reported from all parts of the world (Bowman et al., 2010; Schnieder et al., 2011). Infected dogs can play an important role in the transmission of these two zoonotic nematodes by excreting eggs directly into the human environment. The veterinary and public health aspects of hookworm and *Toxocara* spp. infections in dogs are well established (Bowman et al., 2010; Lee et al., 2010; Overgaauw and van Knapen, 2013). In Europe, there are two hookworm species routinely found in dogs, *A. caninum* and *Uncinaria stenocephala*. *A. caninum* is reported to be found predominantly in dogs located in central and southern Europe (ESCCAP, 2010).

Anthelmintics or combination products with endectocidal activity with increased spectrum of activity can provide the pet owner and veterinarian with the ability to treat dogs that are concurrently infested or infected with multiple parasite types. The efficacy of a combination of spinosad and milbemycin oxime (S/MO) in dogs naturally infected with different species of adult intestinal nematodes has been previously demonstrated in laboratory dose confirmation studies (Schnitzler et al., 2012). More recently it was shown that a minimum dose of 0.75 mg/kg of MO in combination with spinosad will prevent the establishment of the adult stage of the French Heartworm, *Angiostrongylus vasorum* (Böhm et al., 2014). Thus, the studies described below were performed in order to assess the safety and clinical effectiveness of the combination of spinosad and MO (Trifexis®) under field conditions in Europe for the treatment and prevention of naturally acquired flea infestations and the treatment of intestinal nematode infections in pet dogs as compared to authorised control veterinary products (CVP).

2. Materials and methods

2.1. Study design

Two separate randomised, blinded, multicentre field trials were conducted. The dogs enrolled in each field study represented a number of climatic regions as well as a mixture of genders, ages, weights, and dog breeds. The flea clinical study was conducted from April to July 2011 and was a multi-site clinical study in single- and multi-dog (maximum of 4 dogs per household and all received the same treatment) households located predominantly in suburban areas under field conditions in France, in two geographical distinct areas, central (5 sites) and southern (6 sites) France. In addition a maximum of three cats were allowed per household. The study involved a blocked randomisation of households to one of two treatment groups. The order of presentation at the clinic was used for blocking. Eligible dogs at a given site were randomly assigned to one of two treatment groups in the order of presentation to the clinic. Each site's random allocation tables provided for enrolment in a 2:1 ratio for the SMO and SEL groups, respectively. Within single-dog households, sets of three dogs were allocated within each block (two to S/MO and one to SEL). For multi-dog households each block comprised 3 households, two of which were allocated to S/MO and one to SEL. Pet owner, sponsor personnel and contract personnel as well as the treatment technician were unmasked to the treatment; the investigator (and/or personnel performing the whole body flea comb counts) were masked to the treatment. The live phase of the study had a duration of approximately 30 days. All dogs within one household were treated with the same product, either S/MO or SEL, but only dogs with flea counts $\geq$7 fleas on the first visit were included in the subsequent counts on Days 14 and 30. If cats were present in an enrolled household, they also were treated with SEL irrespective of the treatment assigned to the dog(s). During the course of the study three visits were performed: visit 1: dog enrolment and first dosing (in the clinic or at home) on Day 0; visit 2 on Day 14 and visit 3 on Day 30. On all of these visits, physical examinations and whole body flea comb counts were performed and the dogs were also weighed during visits 1 and 3. In addition, owner observations and confirmation of dosing were collected within 3 days of visit 1 via telephone contact with the clinic.

The nematode clinical study was a positive controlled randomised study with a parallel group design conducted with cases recruited from 10 investigational centres (located predominantly in suburban veterinary clinics) in France and 19 in Ireland. Data from both countries were pooled. Dogs were randomised to either the S/MO (Trifexis®) or a (Milbemax; MO in combination with praziquantel; MO/P) group. The randomisation was specific to the site and was unique for single-, and multi-dog households. All nematode positive dogs in the same household received the same treatment. Only those dogs with a positive faecal egg count and which met all of the inclusion criteria and for which none of the exclusion criteria applied were allocated to treatment. These dogs were blocked by consecutive order of enrolment in the study. Each site's random allocation tables provided for enrolment in a 2:1 ratio for the S/MO and MO/P groups, respectively. Within single dog households, sets of three dogs with positive faecal egg counts were allocated within each block (two to S/MO and one to MO/P). For multi-dog households, when more than one dog within a household was positive for faecal eggs at screening, all positive dogs in the household received the same treatment. This was to mitigate the risk of treatment with the wrong product. Dogs from multi-dog households had to be dosed on the same Day 0 with the same treatment. For multi-dog households with more