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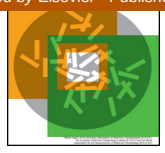

Field evaluation of the efficacy and safety of a combination of spinosad and milbemycin oxime in the treatment and prevention of naturally acquired flea infestations and treatment of intestinal nematode infections in dogs in Europe

Brad Hayes[a], Beate Schnitzler[a], Scott Wiseman[a], Daniel E. Snyder[b,*]

[a] Elanco Animal Health, Eli Lilly and Company Limited, Basingstoke, Hampshire, United Kingdom
[b] Elanco Animal Health Research and Development, a Division of Eli Lilly and Company, 2500 Innovation Way, Greenfield, IN, USA

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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.

* Corresponding author. Tel.: +1 317 277 4439; fax: +1 317 277 4167.
E-mail address: snyder_daniel_e@elanco.com (D.E. Snyder).

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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

than one positive dog, each block comprised 3 households, two of which were allocated to S/MO and one to MO/P. If the household consisted of multiple dogs but only one was positive for faecal eggs at screening, the dog was allocated per the single-dog household list. A single reference laboratory was selected (Charles River Laboratories, Ireland) to carry out all faecal examinations for inclusion of dogs into the study and for examinations performed post-treatment. Initial screening faecal samples were assessed primarily to confirm the presence of *T. canis*, *Toxascaris leonina*, *Trichuris vulpis*, *A. caninum* and *U. stenocephala.* A positive result at screening (i.e. >0 parasite eggs from *T. canis*, *T. leonina*, *T. vulpis* or *A. caninum* identified) enabled the dog to proceed to Day 0 sampling and dosing.

Both studies were conducted in compliance with the Committee for Veterinary Medicinal Products European Union Note for Guidance "Good Clinical Practice for Conduct of Clinical Trials for Veterinary Medical Products' (July 2001) in order to follow the EMA CVMP guidelines for the testing of antiparasitic substances against ticks and fleas. Informed consent was obtained from the dogs' owners before enrolment.

2.2. Selection of animals

In both field studies, client owned dogs of any breed and sex, aged more than 2 months and suspected to have a natural flea infestation and/or an intestinal nematode infection were examined and if they met all relevant inclusion criteria, were selected for enrolment. The primary exclusion criteria included dogs less than 8 weeks of age; dogs <2.0 kg body weight; dogs enrolled in another clinical study; pregnant or nursing bitches, or bitches that were intended for breeding use within 2 months of enrolment; unweaned puppies; dogs that had travelled outside of the EU in the last year; dogs that had been treated with any flea control product with ongoing residual efficacy as per label; dogs treated with drugs active against gastrointestinal nematodes in the preceding 3 months; dogs with pre-existing medical and/or surgical conditions that would impact the collected study data; and dogs that were owned by the investigator, one of the participating hospitals/clinics veterinarians or staff or a family member thereof.

2.3. Treatments

To assess the clinical effectiveness and safety of S/MO (Trifexis®), enrolled dogs were given a flavoured combination tablet of spinosad/milbemycin oxime. The S/MO tablets were administered orally at a dose rate of 45–70 mg spinosad and 0.75–1.17 mg MO/kg bodyweight. To ensure maximum absorption of each drug, S/MO treated dogs were instructed to be fed around the time of dosing. The marketed CVP products selected and used in the flea and nematode field studies were SEL because of its month long adult flea claim and MO/P because of its broad spectrum intestinal nematode efficacy, respectively. Each of these control products was administered as per label instructions from the manufacturer including giving food around the time of dosing.

2.4. Efficacy and safety assessment

The effectiveness of the S/MO combination and SEL were assessed through the reduction in natural flea burdens based on the Day 14 and Day 30 flea counts as compared to the baseline pre-treatment infestation level. The flea counts were log-transformed, statistically modelled and reductions based on model geometric means (GM) were compared between the two treatment groups in order to assess non-inferiority of the S/MO combination as compared to SEL.

In the nematode field study, the primary efficacy parameter was the % reduction in GM faecal egg counts (eggs/g; EPG) between the pre-treatment sample and the post-treatment sample. This was performed separately for each of the four parasites of interest in this study (*T. canis*, *T. leonina*, *T. vulpis* and *A. caninum*). A secondary efficacy parameter was the reduction in GM *U. stenocephala* faecal EPG counts since previous experimentally induced infections in laboratory dose confirmation studies with the S/MO combination had not given 90% or above efficacy. The EPG counts were log-transformed, statistically modelled and the reductions based on model GM were compared between the two treatment groups in order to assess non-inferiority of the S/MO combination as compared to MO/P.

In the flea field study, trained, masked individuals at each site performed whole body flea comb counts during the course of the study. One extra-fine-tooth flea comb was used for each case. Only viable fleas (demonstrating normal movement and behaviour) were counted. Dogs were combed continually for at least 5 min or until no more fleas were found for 5 min.

In the nematode field study, all faecal samples (screening, Day 0 and Day 8 samples) were sent via courier or postal service to a reference testing facility, Charles River Laboratory in Ireland. Freshly collected faecal samples from each dog were shipped ambient or chilled with ice packs, and were stored in a refrigerator prior to shipment and upon receipt. Nematode infections were assessed at the reference laboratory by suitably qualified personnel, who were blinded to the allocation of animals to treatment. Fresh faecal samples for assessment of *T. canis*, *T. leonina*, *T. vulpis*, or *A. caninum* (and *U. stenocephala*, if present) infections were taken from dogs 7 to 14 days prior to Day 0 (screening), on Day 0 prior to treatment (to confirm ongoing infections) and again on Day 8. All EPG counts were carried out by the reference laboratory using the same methods. For the screening faecal examination, the McMaster method was used. Briefly, 4 g of faeces were suspended in 56 mL of a sugar flotation solution (sp. Grav. > 1.2) and processed and examined as per the McMaster technique (Zajac and Conboy, 2006). For the Day 0 prior to treatment and the Day 8 post-treatment faecal examinations, a direct faecal double centrifugation flotation method was used. Briefly, 1–2 g ± 0.1 g of fresh faeces were weighed and mixed with 20 ± 1 mL water and thoroughly mixed and processed using published methods (Zajac and Conboy, 2006). During the examination of each faecal sample, the number of eggs counted for each of the five nematode species of interest was divided by the number of grams of faeces weighed out and used to determine the EPG of each

nematode species for each individual dog. Differentiation of hookworm eggs was based on the size of the eggs and the experience of the parasitologist at the reference laboratory.

The safety of S/MO combination in each field study was evaluated through review of adverse events and changes in body weight during the study.

2.5. Statistical analysis

These data were skewed in both studies irrespective of treatment group, particularly at the pre-treatment time point; therefore, log transforming the counts for analysis was appropriate. The use of GM was appropriate in highly skewed data distributions to estimate the centre of the data distribution and permit valid statistical analysis.

Flea count data were log-transformed and analysed using repeated measures mixed model methodology. The correlation between observations on the same dog and between dogs within the same household was accounted for in the model.

The reduction in flea count was calculated using back-transformed model LS means with the following equation:

$$\%\text{Reduction} = \left(\frac{\text{Geometric mean}_{\text{pre-treatment}} - \text{Geometric mean}_{\text{post-treatment}}}{\text{Geometric mean}_{\text{pre-treatment}}} \right) \times 100.$$

Non-inferiority of the S/MO group in relation to SEL was declared if both products were effective as defined by statistically significant reduction of the flea infestation by ≥90%.

The treatment difference and corresponding 95% confidence interval in the proportion of dogs successfully treated was calculated. Success in an individual dog was defined as a reduction of at least 90% in flea count post-treatment compared to the pre-treatment count.

The primary efficacy parameter in the nematode study was the % reduction in GM faecal egg counts between the pre-treatment sample and the post-treatment sample. This was performed separately for each of the four parasites of interest in this study (*T. canis*, *T. leonina*, *T. vulpis* and *A. caninum*). Secondary efficacy parameters were the reduction in GM *U. stenocephala* faecal EPG counts and the reduction in total infection (total infection was defined as the sum of the egg counts from all 5 species enumerated.)

For each of the primary and secondary efficacy variables the faecal egg count data were analysed using repeated measures mixed models, accounting for the correlation between observations on the same dog and between dogs within the same household. The same criteria were used to assess non-inferiority and calculate success rates as in the flea count data assessment.

The reduction in egg count was calculated using the following equation:

$$\%\text{Reduction} = \left(\frac{\text{Geometric mean}_{\text{pre-treatment}} - \text{Geometric mean}_{\text{post-treatment}}}{\text{Geometric mean}_{\text{pre-treatment}}} \right) \times 100$$

Data from small individual study sites (<4 households with efficacy evaluable cases) were pooled for analysis within geographical region. In instances where, after initial pooling within region (i.e. central versus southern France) there remained sites with <4 households with efficacy evaluable data, pooling also occurred across regions.

For each of the primary and secondary variables, the proportion of dogs successfully treated was also calculated; success in an individual dog was defined as a reduction of at least 90% in faecal egg count post-treatment compared to the pre-treatment count.

3. Results

Eleven veterinary clinics situated in geographically diverse regions of France participated in the flea study. 54.5% of S/MO cases and 53.4% of SEL cases were located in central France and the remainder in southern France. In total, 176 S/MO treated dogs and 88 SEL treated dogs remained on study through to the final visit at Day 30. A total of 150 S/MO treated dogs and 71 SEL treated dogs were included in the effectiveness evaluable population. Ten sites contributed data to the effectiveness evaluable population; One site (site 10) was excluded as it had <4 households and although eligible for pooling, there were no other sites in the region to pool this site with. Site 7 included the largest number of cases in the effectiveness evaluable population (28 S/MO and 13 SEL cases).

The primary objective of the study was to demonstrate non-inferiority of S/MO in combination when compared to SEL. During the course of this study, the S/MO treated dogs demonstrated >95% reductions at both post-baseline flea count assessments based on geometric means (Table 1). Non-inferiority between treatments was demonstrated. The success rates for S/MO were 96.7% and 88.7%, on Days 14 and 30, respectively, compared with 85.9%, and 73.2% for the SEL.

The number of dogs (percent) with no live fleas in the S/MO-treated group at Days 14 and 30 were 89.3% and 80.0%, respectively (Table 2). The number of dogs (percent) with no live fleas in the SEL group at Days 14 and 30 were 77.5% and 70.4%, respectively.

Prior to treatment on Day 0 the GM number of fleas combed from the 150 effectiveness evaluable dogs in the S/MO group was 16.4 (range 7–175). In the SEL group the GM was 12.0 (range 7–47) (Table 1). By Day 14 the GM flea counts were significantly ($p < 0.001$) reduced in both groups to 0.2 in the S/MO group and 0.3 in the SEL group (Table 1). In the S/MO group the reduction was 98.97% and for SEL the corresponding reduction was 97.43%. The level of reduction in flea burden remained significant ($p < 0.001$) in both groups at Day 30. In the S/MO group the

Table 1
Geometric mean flea counts and percent reductions (as compared to baseline) in IVP and CVP treatment groups.

Effectiveness	Baseline geometric mean flea count (range)	Geometric mean flea count (% reduction)	
		Day 14	Day 30
Spinosad/MO (IVP)	16.4 (7–175)	0.2 (98.97)	0.4 (97.37)
Selamectin (CVP)	12.0 (7–47)	0.3 (97.43)	0.7 (93.96)

Table 2
Percentage of f dogs with no live fleas in the IVP and CVP treated groups at Days 14 and 30 post-treatment.

	Percent dogs with no live fleas	
	Day 14	Day 30
Spinosad/MO (IVP)	89.3	80.0
Selamectin (CVP)	77.5	70.4

reduction was 97.37% (GM = 0.4) and for SEL the corresponding reduction was 93.96% (GM = 0.7). Comparison of the treatment groups showed no significant difference between the treatments at either Day 14 ($p = 0.180$) or Day 30 ($p = 0.148$). Success rates, defined as ≥90% reduction in flea count compared to pre-treatment for an individual dog in the S/MO group was 96.7% with 89.3% of dogs with no live fleas. This compares favourably with the SEL group where the success rate was 85.9% with 77.5% of dogs with no live fleas. The mean difference in success rates at Day 14 was 11% and was significant based on the 95% confidence interval for the difference (2–19%). At Day 30 the success rate in the S/MO group was 88.7% with 80.0% of dogs with no live fleas and the success rate in the SEL group was 73.2% (70.4% with no live fleas). The difference in success rates at Day 30 was also significant based on the 95% confidence interval (4–27%).

In total 10 adverse events (AEs) were observed in seven S/MO treated dogs in the flea field study. Overall, the highest frequency AE was emesis during the course of the study (animal rate 2.2%, incidence 2.2%) and starting on the day of or day following dosing with 3 events in the S/MO group. In the SEL group there were two AEs, both in the same dog.

Of the 2333 dogs screened for *T. canis*, *T. leonina*, *T. vulpis and A. caninum* eggs in the nematode field study, 238 dogs were found to be positive, with 229 subsequently randomised onto the study. Of these 229 dogs, 151 were treated with a single dose of S/MO, and 77 were treated with a single dose of MO/P. The nematode species present on Day 0 were well balanced between the two treatment

Table 3
Geometric mean (GM), percentage reduction and p-value of faecal egg counts (EPG) of nematode parasites in dogs treated with a combination of spinosad/milbemycin oxime or a control veterinary product (CVP).

Nematode type	Baseline pre-treatment GM faecal EPG counts		Post-treatment GM faecal EPG counts[a,b]	
	Spinosad/MO	CVP	Spinosad/MO	CVP
Toxocara canis				
EPG GM	42.0	27.4	0.4	0.9
% Reduction			99.06% (*N* = 53)	96.68% (*N* = 26)
p-value[c]			<0.001	<0.001
Toxascaris leonina				
EPG GM	19.0	15.3	0.0	0.6
% Reduction			99.5% (*N* = 5)	96.39% (*N* = 2)
p-value[c]			0.008	0.085
Trichuris vulpis				
EPG GM	72.5	80.2	0.9	1.0
% Reduction			98.79% (*N* = 70)	98.76% (*N* = 39)
p-value[c]			<0.001	<0.001
Ancylostoma caninum				
EPG GM	67.0	92.3	0.0	0.3
% Reduction			99.98% (*N* = 45)	99.65% (*N* = 22)
p-value[c]			<0.001	<0.001
Uncinaria stenocephala				
EPG GM	44.2	25.1	3.2	5.0
% Reduction			92.8% (*N* = 35)	79.9% (*N* = 23)
p-value[c]			<0.001	<0.001
All nematodes				
EPG GM	94.0	93.9	1.3	2.3
% Reduction			98.57% (*N* = 134)	97.57% (*N* = 73)
p-value[c]			<0.001	<0.001

[a] Geometric mean = exp (LS mean) − 1, which used LS mean values from the mixed model analysis, where LS mean = least squares mean.
[b] Percent reduction in geometric mean = 100 × ((geometric mean for Day 0 visit 1 − geometric mean for post-treatment Day 8 visit 2)/geometric mean for Day 0 visit 1).
[c] p-value for the difference between pre- and post-treatment faecal egg count within treatment group.

Table 4
Success rates of post-treatment faecal egg count reduction by type of nematode and for all nematodes.

Nematode type	Treatment	Success[a]	Comparison	Difference in success rates[b] (%)	95%CI[c]
T. canis	Spinosad and MO ($N=53$) Milbemax ($N=26$)	50 (94.3%) 23 (88.5%)	Spinosad/MO – Milbemax	6	8–20
T. leonina	Spinosad and MO ($N=5$) Milbemax ($N=2$)	5 (100.0%) 2 (100.0%)	Spinosad/MO – Milbemax	0	0–0
T. vulpis	Spinosad and MO ($N=70$) Milbemax ($N=39$)	63 (90.0%) 35 (89.7%)	Spinosad/MO – Milbemax	0	−12 to 12
A. caninum	Spinosad and MO ($N=45$) Milbemax ($N=22$)	45 (100.0%) 20 (90.9%)	Spinosad/MO – Milbemax	9	−3 to 21
U. stenocephala	Spinosad and MO ($N=35$) Milbemax ($N=23$)	23 (65.7%) 11 (47.8%)	Spinosad/MO – Milbemax	18	−8 to 44
All nematodes	Spinosad and MO ($N=134$) Milbemax ($N=73$)	116 (86.6%) 58 (79.5%)	Spinosad/MO – Milbemax	7	−4 to 18

[a] Success was the number of dogs with ≥90% individual percent reduction in faecal egg count. Individual percent reduction was calculated using 100 × ((Day 0 visit 1 pre-treatment faecal egg count − Day 8 visit 2 post-treatment faecal egg count)/Day 0 visit 1 pre-treatment faecal count).
[b] Success rate was the proportion of dogs in a treatment group for which treatment was successful.
[c] 95% confidence interval (CI) was based on the normal approximation to the binomial distribution.

groups. 56.0% of dogs in the S/MO group had single infections versus 56.2% in the MO/P group. The remaining dogs had mixed infections with 2–4 nematode species. The geographical distribution of cases in the effectiveness evaluable population shows that 28.4% of the dogs in the S/MO group and 30.1% of the dogs in the MO/P group were from Ireland. The remaining 71.6% (S/MO) and 69.9% (MO/P) of dogs were from France. However, there was some variation in the distribution of nematode types. *T. canis* was relatively evenly distributed between Ireland and France with 58.5% (IVP) and 69.2% (S/MO) of *T. canis* cases from Ireland. *T. leonina* was found exclusively in Ireland ($n=7$), *T. vulpis* was found predominantly in France with 95.7% (S/MO) and 89.7% (MO/P) of dogs with *T. vulpis* from France. *U. stenocephala* was found in Ireland and France in the ratio expected for the geographical distribution of cases with 71.4% (S/MO) and 78.3% (MO/P) in France. *A. caninum* was found exclusively in France ($n=67$).

Percent reductions in post-treatment geometric mean faecal egg counts were performed separately for each of the four parasites of primary interest in this study (*T. canis*, *T. leonina*, *T. vulpis* and *A. caninum*), and for the secondary parameters of *U. stenocephala* and the reduction in total infection, 'all nematodes' (Tables 3 and 4).

For the safety assessment aspect of this nematode field study, 3 from 151 dosed animals in the S/MO group (2.0%) reported an adverse event (AE), 2 of which were diarrhoea and 1 of which was dermatitis/eczema. From the MO/P group, 2 from 77 dosed animals (2.6%) reported an AE, both of which were diarrhoea. No AEs started on the day of or day following dosing and there was no emesis observed.

4. Discussion

In both randomised, blinded, CVP controlled multicentre European field trials, the combination of S/MO for the treatment of natural flea infestations as well as adult intestinal nematode infections was highly successful as compared to positive reference CVPs. The cat flea is considered the most common insect ectoparasite of dogs in Europe (Halos et al., 2014). In the face of high prevalence of the cat flea that has both pathogenic and vector potential, effective integrated adult flea control represents a major and ongoing objective in small-animal veterinary practices. In the nematode field study, dogs enrolled with positive faecal egg counts were also found to be infested with fleas (35.1–38.4% in both treatment groups), so the routine use of a broad spectrum endectocide like Trifexis® will control multiple endo- and ectoparasites in dogs when used by pet owners. A large number of dogs were screened ($N=2339$) to identify dogs positive ($N=238$) for nematode eggs; however, these results demonstrate that worm infections are common (ca. 10% in this study) in dogs in Europe. This prevalence is similar to what other authors have found in dog faecal surveys conducted in Europe (Schnieder et al., 2011). The most commonly found intestinal nematode species seen in this field study, either alone or in combination, including the zoonotic parasites *T. canis* and *A. caninum*, demonstrate the need to routinely conduct faecal examinations and to treat client owned dogs for these different intestinal nematode parasites. In a number of dogs in this study, Day 0 pre-treatment faecal egg count numbers were very high, establishing the high risk of environmental contamination by non-treated, infected dogs in home environments. The zoonotic potential of *T. canis* and *A. caninum* are well known in the scientific literature, including in Europe (Bowman et al., 2010; Deplazes et al., 2011; Lee et al., 2010; Overgaauw and van Knapen, 2013). Schnieder et al. (2011) summarised the prevalence of *T. canis* in several European countries. Human exposure based on seroprevalance to *T. canis* has also been reported in different regions of the world where the age adjusted *Toxocara* seroprevalence was 13.9% (Won et al., 2008; Manini et al., 2012). Macpherson (2013) reviewed the most common control measures for *T. canis*, including regular and frequent anthelmintic treatment of dogs starting at an early age, education and enforcement of

laws for the disposal of canine faeces, dog legislation and personal hygiene. Since *T. canis* eggs are very environmentally resistant and can survive well over most winters in temperate climates, reducing environmental contamination from infected dogs is very important. Additionally, *T. canis* can be routinely found in both juvenile and mature dogs (Fahrion et al., 2008), so routinely monitoring and treating of all age classes of dogs is important.

The availability of broad spectrum endectocides to pet owners that effectively treat dogs with adult intestinal nematodes helps to address recommendations made to veterinarians (Epe, 2009) and recommendations by expert groups such as ESCCAP (2010) to routinely treat and control all intestinal nematodes. This is of particular importance for zoonotic species like *T. canis* and *A. caninum* that have been shown to be consistently found in dogs of all ages and throughout the year in prevalence studies conducted in different areas of the world, including healthy, well-cared for dogs in Europe (Martinez-Moreno et al., 2007; Riggio et al., 2013; Neves et al., 2014). ESCCAP (2010) points out that there is surprisingly little information about the impact of re-treatment intervals on parasite burdens and environmental contamination on which to base a maximum re-treatment interval. However current information suggests annual or twice yearly treatments do not have a significant impact on preventing patent infection within a population of dogs, so a treatment frequency of at least 4 times per year is a general recommendation. ESCCAP (2010) further states that it has been shown that an increase in treatment frequency effectively reduces the occurrence of positive animals; studies have shown that deworming treatments four times a year does not necessarily eliminate patent infections, while monthly deworming treatments can largely prevent patent infections as it takes into account the biology of these intestinal nematode parasites. This is exemplified in a recent publication that demonstrated that the prevalence of common nematode and cestode endoparasites has declined significantly in a population of well-cared for dogs that were analysed and was attributed to the monthly use of broad spectrum endectocides (Gates and Nolan, 2014). The highly significant reduction (92.8%) in *U. stenocephala* egg counts indicates that the combination of S/MO is additionally reducing or affecting egg laying of adult worm populations of this hookworm species, possibly due to the higher MO minimum dose (0.75 mg/kg) as compared to Milbemax, even though there is not a specific label claim for this parasite on the European Trifexis® label.

In conclusion and based on the results presented above for both field studies, the combination of spinosad and milbemycin oxime was shown to be a safe and effective product for the treatment and prevention of fleas (*C. felis*) and the treatment of commonly found intestinal nematodes of dogs in Europe.

Conflict of interest

The studies as reported herein were funded by Elanco Animal Health. The authors are current employees of Elanco Animal Health and assisted with the study design, study conduct, data analysis, and review of the manuscript; however, there were no conflicting interests that may have biased the work reported in this paper.

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