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Research paper

The sustained speed of kill of ticks (*Rhipicephalus sanguineus*) and fleas (*Ctenocephalides felis felis*) on dogs by a spot-on combination of fipronil and permethrin (Effitix®) compared with oral afoxolaner (NexGard®)

Dejan Cvejić[a], Claudia Schneider[a], Willem Neethling[b], Klaus Hellmann[a], Julian Liebenberg[b], Christelle Navarro[c,*]

[a] Klifovet AG, Geyerspergerstr. 27, D-80689, München, Germany
[b] ClinVet International (pty) Ltd.,Uitsigweg, Bainsvlei, 9338 Bloemfontein, South Africa
[c] Virbac SA, BP27, 06511 Carros Cedex, France

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ABSTRACT

The rapid speed of kill of a spot-on, combination of fipronil-permethrin (Effitix®, Virbac) was shown against infestations of *Rhipicephalus sanguineus* and *Ctenocephalides felis* on dogs. Efficacy was determined against new infestations at weekly intervals for one month after treatment.

Dogs were allocated randomly to either an untreated control or to a single administration, given on Day 0, of either topical fipronil-permethrin (6.7–13.4 mg/kg and 60–120 mg/kg, respectively) or oral afoxolaner (2.72–6.8 mg/kg), based on pre-treatment, host-suitability flea counts. Dogs were infested with 50, unfed, adult *R. sanguineus* on Days 7, 14, 21 and 28, and with 100*C. felis* on Days 8, 15, 22 and 29. Tick counts were performed 0.5, 2, 6, 12 and 24 h, and flea counts were performed 0.5 and 24 h after each infestation.

No treatment-related adverse reactions occurred. Dogs in the untreated group maintained viable infestations throughout the study. Following infestation, live tick and flea counts for dogs treated with fipronil-permethrin compared with untreated dogs were rapidly and significantly reduced with efficacy apparent at 0.5 h after infestation. Flea efficacies (arithmetic mean counts) at 0.5 h after infestation on Day 7 (Day 28) were significantly greater for fipronil-permethrin, 70% (34%) compared with 8% (18%) for afoxolaner ($P \leq 0.05$). Tick efficacies at 2 h on Day 7 (Day 28) were 74% (63%) for fipronil-permethrin compared with 10% (0%) for afoxolaner ($P \leq 0.05$). Efficacies for tick repellency as indicated by counts of ticks off the dogs at 2 h on Day 7 (Day 28) were greater for fipronil-permethrin, 32% (22%) compared with afoxolaner, 0% (0%) ($P \leq 0.05$). Anti-attachment efficacies at 12 h were greater for fipronil-permethrin compared with afoxolaner. Tick efficacies at 24 h, based on arithmetic (geometric) means, were significantly greater on Day 28 for fipronil-permethrin compared with afoxolaner ($P \leq 0.05$), 74% (87%) and 45% (60%), respectively, and were similar ($P > 0.05$) on Days 7, 14 and 21. Flea efficacies, 24 h after infestation were > 98% and similar for both treated groups on all infestation days ($P > 0.05$).

The topically applied fipronil-permethrin containing ectoparasiticide Effitix® offers rapid efficacy against *R. sanguineus* and *C. felis* which persists for one month after a single administration in dogs. Afoxolaner is also effective although speed of kill is slower. The rapid and sustained speed of kill of both parasites by fipronil-permethrin should contribute to effective management not only of these parasites and their direct adverse effects including irritancy and allergy, but also to reducing the risk of transmitting infections.

1. Introduction

Treatment and prevention of ectoparasite infestations in dogs usually requires occasional or regular use of drugs with the ability to repel and/or kill the specific parasites without causing concern for safety. Canine vector-borne diseases comprise a group of globally distributed and spreading illnesses that are caused by a wide range of pathogens transmitted by arthropods (Otranto et al., 2009a, 2009b). In addition to their veterinary importance, many of these canine vector-borne pathogens may also affect the human population due to their zoonotic potential, a situation that requires a 'One Health' approach (Maia et al., 2015; Mencke et al., 2013). It is therefore of paramount

* Corresponding author.
E-mail address: christelle.navarro@virbac.fr (C. Navarro).

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importance, that products that are administered to prevent, control or treat ticks and fleas in dogs, kill such parasites prior to transmitting disease and therefore have a very fast speed of kill.

The cat flea, *Ctenocephalides felis felis,* is the major ectoparasite of dogs and cats and is found endemic and worldwide (Rust and Dryden, 1997). Adult fleas are the only life stage usually found on these hosts as eggs fall off the animals' coat, hatch and the larvae and pupae develop in the environment (Dryden, 1989 Krämer and Menke, 2001). Adult fleas spend most of their life on the host, begin mating and about 24 h after the first blood meal, females start to lay eggs (Dryden and Broce, 2002). Effective flea control is dependent on elimination of fleas from the animal and its environment (Rust and Dryden, 1997). This may be achieved using a flea adulticide in combination with an insect growth regulator that disrupts the development of eggs and/or larvae (Dryden and Broce, 2002; Chin et al., 2005). Fleas are a major cause of allergic skin disease in dogs and cats, and in large numbers these blood-feeders may cause anaemia (Krämer and Menke, 2001).

Ticks are endemic in many regions including Europe and North America. In Europe, there are more than 12 species with varying biology and geographical distribution and three of the most common species which commonly infest dogs are *Rhipicephalus sanguineus* sensu lato*, Ixodes ricinus*, and *Dermacentor reticulatus* (Beugnet and Marie, 2009). The brown dog tick, *R. sanguineus* is one of the most widely distributed tick species, occurring globally between 35°S and 50°N, and it shows a preference for feeding on dogs for all stages of its lifecycle, and unusually for ticks, thrives in home and kennel environments (Dantas-Torres, 2008). Tick infestations of dogs may vary from an occasional single infestation to continuous and high infestations, and can cause serious and in some cases life-threatening disease (Dryden and Pain, 2004). Direct harm from *R. sanguineus* may arise through irritation produced during attachment and feeding on the host, and by causing anaemia in large infestations (Bowman, 2008). This tick is also a vector of several pathogens which may, in some cases cause severe and life-threatening clinical disease in dogs and humans, for example *Ehrlichia canis* (the cause of canine monocytic ehrlichiosis), *Babesia vogeli, Hepatozoon canis* (if ingested), Rickettsial infections such as *Rickettsia conorii* and *R. rickettsia* the causes of Mediteranean spotted fever and Rocky Mountain spotted fever, respectively, and some *Anaplasma* species (Otranto and Dantas-Torres, 2010; Mencke, 2013). The transmission of pathogens by ticks is believed to occur typically within 24–48 h of infestation and attachment. However, more recent evidence suggest that much earlier transmission is possible, for example transmission of *E. canis* by *R. sanguineus* may occur from 3 h after attachment (Fourie et al., 2013a). Therefore, effective and very fast-acting acaricides are important to not only reduce irritation produced directly by ticks but also are of particular importance to minimise the risk of pathogen transmission (Davoust et al., 2003) especially, as other methods of preventing or treating clinical disease caused by the transmitted pathogens are limited. Acaricides are required to act either as a repellent avoiding attachment of the parasite on the host and/or may rapidly kill the parasite after a blood meal; beyond killing the parasite, the treatment should also aid to reduce the risk of transmission of infection, particularly in the case of *E. canis* (Fourie et al., 2013a,b).

Control of fleas and ticks is based principally on the use of parasiticides and the most widely used products are effective against both fleas and ticks. Various delivery systems and formulations are available including collars, spot-on's, sprays and oral formulations (Beugnet and Franc, 2012). Treatment of dogs with spot-on formulations provides convenience of use and, typically, a monthly dosing interval will provide efficacy that is both curative (i.e. ability to kill ticks and fleas when given to an infested dog) and preventive (i.e. ability to prevent tick infestations for one-month after treatment administration). Fipronil and permethrin used topically have been shown to be very effective against ticks (Dryden and Payne, 2004) and fleas, and another combination of actives with early repellent, anti-attachment and killing effects (fipronil, amitraz, (*S*)-methoprene) was shown to be effective as an acaricide and in preventing transmission of canine monocytic ehrlichiosis (Davoust et al., 2003; Otranto et al., 2008; Fourie et al., 2013a,b). The combination of fipronil-permethrin has recently been reported to be highly effective for one month against fleas and ticks (Bonneau et al., 2015a,b,c). Afoxolaner given orally has also been shown to be effective in the treatment and prevention of tick and flea infestations (Kunkle et al., 2014a,b).

The study reported here describes the results of an experiment in dogs that compared the acaricidal-insecticidal efficacy and the sustainable speed-of-kill of a topical spot-on formulation of fipronil and permethrin with that of either an untreated control or oral afoxolaner in the prevention of infestation by the most common species of tick, *R. sanguineus*, and flea, *C. felis*. While permethrin acts as a repellent, topically applied permethrin and fipronil also act as a contact acaricide. Afoxolaner, given orally, needs a blood meal to be taken by the parasite on the host in order to kill it. Differences in administration route and mode of action between products may result in differences in the speed of onset of their activity which is especially relevant to prevention of infestation by the parasite and potentially allowing the transmission of vector-borne disease pathogens to the host. The speed of kill is of paramount of importance to evaluating the efficacy products claiming that they can both prevent and treat tick and flea infestations. Therefore, this study compares two products for their ability to rapidly kill or avoid attachment of tick and fleas, and thereby evaluate their potential use in limiting the transmission of pathogens.

2. Materials and methods

The study was approved by the ClinVet independent ethics and animal welfare review (CV 15/209), and was in compliance with South African animal welfare regulations. The study followed the European Medicines Agency Guidelines for the testing and evaluation of antiparasitic substances for the treatment and prevention of tick and flea infestations in dogs and cats (EMEA, 2007) and was a GCP study (EMEA, 2000). The positive control product, afoxolaner (NexGard®) was chosen as it, like fipronil-permethrin (Effitix®), is indicated for the treatment and prevention of flea and tick infestations on dogs for at least one month.

2.1. Animals, housing and management

Eighteen, healthy, purpose-bred, mongrel and Beagle dogs were selected from 22 dogs on the basis of the highest, pre-treatment flea counts. The dogs had not been treated with an ectoparasiticide for at least 12 weeks, had shown good tick retention prior to treatment and were in good health at enrolment. The seven male and 11 female dogs weighed between 10 and 20 kg (except for one dog, allocated randomly to the untreated control group that weighted 24.4 kg), were 14–97 months of age, hair length was between 16 and 29 mm, and the females were confirmed as not pregnant. Each dog was uniquely identified, fed once daily a commercial diet and had free access to water. Dogs were housed individually in cages with concrete floors and an elevated stainless steel mesh bench to rest; temperature and humidity were ambient.

2.2. Study methods

The dogs were acclimatised for seven days prior to Day 0, defined as the day that dogs were administered treatments. The 22 dogs were controlled to be free of fleas and ticks, before being infested with fleas on Day −6. Flea counts were done on Day −5, which served to select 18 dogs with the highest flea counts. Those were ranked, within sex, and fleas counts in descending order and randomly allocated to the three treatment groups: untreated negative control, fipronil-permethrin spot-on topical treatment and afoxolaner oral treatment. Body weights were determined on Day −4 for treatment. The fipronil-permethrin

treatment was given according to the Effitix® product label as a single pipette that provided a dose range of 6.7–13.4 mg fipronil/kg and 60–120 mg permethrin/kg depending on the weight of the animal. The contents were placed on the skin at a minimum of two locations on the dorsal surface. Care was taken to avoid spillages and no run-off of the product occurred. The afoxolaner treatment was given orally according to the product label for NexGard® 68 mg as a single unit dose, chewable tablet containing 68 mg afoxolaner as recommended for dogs of 10–25 kg body weight, equivalent to 2.72–6.8 mg afoxolaner/kg. General health observations were performed on each dog at least once a day from Day −7, and each dog was given a veterinary examination on Day −7 and −1 to evaluate general health and confirm suitability for inclusion in the study.

For tick infestation, 25 male and 25 female adult, *R. sanguineus* (recent strain originating from France) at least 7 days-old and unfed were applied to each animal. To allow attachment, dogs were restrained for 10 min during which any ticks that dropped off were re-placed. The animals were then confined in infestation crates for 2 h. Tick infestations were made on Days 7, 14, 21 and 28. Tick counts were made *in situ* by trained staff using palpation and direct observation with systematic parting of the entire hair-coat on each dog. The exact time of each tick count was determined for each individual dog to ensure that it was as close as possible to the intended time. Tick counts were made at 0.5, 2, 6, 12 and 24 h after each infestation. After each of the 24 h counts, the ticks were removed from the animals and all animals combed to ensure no ticks were missed. At each count, ticks were classified individually as either live or dead, and as either free, attached and un-engorged (absence of filling of the alloscutum) or attached and engorged. Live ticks were defined as exhibiting normal behaviour and capable of co-ordinated movement on external stimulation. For the 0.5 and 2 h tick counts on Days 7, 14, 21 and 28, the ticks that had been repelled or fallen off the host and were in the infestation crates were collected, counted and classified.

For flea infestations, a pre-counted aliquot of approximately 100, mixed sex, adult *C. felis* (European source) were placed on each of the dogs on Days 8, 15, 22 and 29 (after tick removal). Parasites were placed on the animals distant to the site of treatment administration. Whole body, flea counts were made at 0.5 h after each infestation by removing fleas and re-infesting all fleas after counting for each animal. Fleas were removed and counted using a fine-toothed flea comb at 24 h after infestation. The whole body of each animal at each combing was combed at least twice until no further fleas were found. All staff involved in flea and tick counts were blinded.

2.3. Data analysis

The study was of a controlled, randomised and blocked, blinded, parallel group design. The experimental unit was the individual animal. Homogeneity criteria of the pre-treatment populations on Day −4 for body weight and hair length were confirmed to be similar between treatment groups ($P > 0.05$). The objectives of the study for ticks were to determine the persistence of efficacy for repellency, anti-attachment activity and acaricidal speed of kill of each of the treatments against tick infestations by *R. sanguineus*. The objectives of the study for fleas were to determine the persistence and speed of kill, and insecticidal efficacy as determined by live flea counts. The primary efficacy variable for each parasite was defined by arithmetic mean count reductions after infestation in each of the treated groups compared with the count for the corresponding time in the untreated control group. Percent efficacy was calculated using Abbott's formula:

$$\text{Percentefficacy} = 100x\frac{(\text{meancount(untreated)} - \text{meancount(treated)})}{\text{meancount(untreated)}}$$

To determine the repellent (or expellent) efficacy against ticks, population of ticks on the host but unattached was evaluated. For calculation of repellence of ticks based on the total numbers of unattached ticks (live and dead) remaining on the animals at 0.5 and 2 h, the arithmetic mean numbers of ticks on each of the treated groups was compared with the arithmetic mean numbers of ticks on the untreated control group for the corresponding time. Anti-attachment efficacy was calculated using the total counts of attached (live and dead) ticks at 6, 12 and 24 h after each infestation for each of the treated groups compared with the total counts for the corresponding times in the untreated control dogs.

The tick and flea data presented as arithmetic mean counts were used for calculation of percent efficacy, as recommended by EMEA (2007) however, as this study was focused on speed of kill and persistence the final tick counts were made at 24 h, rather than at 48 h after infestation which is typically recommended for acaricidal and insecticidal efficacy. For statistical analyses of between treatment effects, the tick and flea counts were also transformed by the $\log_e$ (count + 1) transformation prior to analysis in order to stabilise the variance and normalise the data. Using the PROC GLM procedure (SAS® 9.2 software programmes (SAS Institute Inc, Cary, North Carolina, USA)), data for the untransformed counts (arithmetic means) and the transformed counts (geometric means) were analysed using a linear ANOVA model for treatment effect. The treatment effects for untransformed counts were finally compared using Mann-Whitney tests for each contrast. Testing was two-sided at the significance level of $\alpha = 0.05$. The results for flea and tick counts were similar using untransformed and transformed counts except that the Mann-Whitney results tended to be the most conservative (i.e. least likely to indicate a significant treatment effect) and therefore these results are reported.

3. Results

3.1. Efficacy

All of the tick infestations were viable and vigorous as indicated

Table 1
Arithmetic mean counts of live (attached and unattached) *Rhipicephalus sanguineus* on dogs, and percentage acaracidal efficacies, for animals untreated or treated with either fipronil-permethrin topically or afoxolaner orally.

Day[1]	Hour[2]	Untreated	Fipronil-Permethrin	Afoxolaner
Day 7	0.5	40.5[a]	26.8[b] (33.7)	40.8[a] (0.0)
	2	36.3[a]	9.3[b] (74.3)	32.7[a] (10.1)
	6	28.7[a]	9.7[b] (66.3)	27.5[a] (4.1)
	12	28.7[a]	3.8[b] (86.6)	22.0[a] (23.3)
	24	25.0[a]	1.0[b] (96.0)	1.2[b] (95.3)
Day 14	0.5	45.5[a]	11.7[b] (74.4)	45.2[a] (0.7)
	2	37.0[a]	4.7[b] (87.4)	32.2[a] (13.1)
	6	29.2[a]	3.7[b] (87.4)	26.8[a] (8.0)
	12	23.7[a]	3.5[b] (85.2)	21.2[a] (10.6)
	24	13.3[a]	1.0[b] (92.5)	1.7[b] (87.5)
Day 21	0.5	42.0[a]	21.8[b] (48.0)	40.8[a] (2.9)
	2	38.8[a]	12.0[b] (69.1)	38.0[a] (2.1)
	6	35.3[a]	8.8[b] (75.0)	32.4[a] (8.3)
	12	29.0[a]	7.8[b] (73.0)	23.8[a] (17.9)
	24	21.2[a]	4.0[b] (81.1)	7.0[b] (66.9)
Day 28	0.5	37.2[a]	22.2[b] (40.4)	43.4[a] (0.0)
	2	34.7[a]	13.0[b] (62.5)	37.2[a] (0.0)
	6	28.0[a]	11.3[b] (59.5)	24.2[a] (13.6)
	12	26.8[a]	6.5[b] (75.8)	21.2[a] (21.0)
	24	18.7[a]	4.8[b] (74.1)	10.2[a] (45.4)

[1] Day of infestation.
[2] Time after infestation. Each dog was infested with 50 ticks on each Day at 0 h.
Percent acaricidal efficacy (live ticks) for either fipronil-permethrin or afoxolaner treatment of dogs relative to untreated dogs is shown in parentheses.
[a,b] Counts within a row are significantly different $P \leq 0.05$ using both untransformed (arithmetic) and transformed (geometric) mean counts.
There were 6 animals per treatment group at each time, expect for the afoxolaner group on Days 21 and 28 for which there were 5 dogs per group.

Table 2
Arithmetic mean counts of total live (*Ctenocephalides felis felis*) on dogs percentage efficacies, for animals untreated or treated with either fipronil-permethrin topically or afoxalaner orally.

Day[1]	Hour[2]	Untreated	Fipronil-Permethrin	Afoxalaner
Day 8	0.5	89.3[a]	27.2[b] (69.6)	82.0[a] (8.2)
	24	74.0[a]	0.0[b] (100.0)	0.0[b] (100.0)
Day 15	0.5	85.0[a]	21.5[b] (74.7)	74.0[a] (12.9)
	24	68.3[a]	0.0[b] (100.0)	0.0[b] (100.0)
Day 22	0.5	91.8[a]	36.8[b] (59.9)	69.8[a]* (24.0)
	24	70.7[a]	0.0[b] (100.0)	0.0[b] (100.0)
Day 29	0.5	83.0[a]	54.7[b] (34.1)	68.2[a] (17.8)
	24	63.8[a]	1.0[b] (98.4)	0.0[b] (100.0)

[1] Day of infestation.
[2] Time after infestation. Each dog was infested with c. 100 fleas on each Day at 0 h.
Percent efficacy for either fipronil-permethrin or afoxalaner treatment of dogs relative to untreated dogs is shown in parentheses.
[a,b] Counts within a row are significantly different $P \leq 0.05$ using both untransformed (arithmetic) and transformed (geometric) mean counts, except for * which for transformed counts was not significantly different from untreated control.
There were 6 animals per treatment group at each time, expect for the afoxalaner group on Days 22, 23, 29 and 30 for which there were 5 dogs per group.

Table 3
Arithmetic mean counts of total unattached (live and dead) *Rhipicephalus sanguineus* repelled but remaining on dogs, and repellent (on-dog) percentage efficacies, for animals untreated or treated with either fipronil-permethrin topically or afoxolaner orally.

Day[1]	Hour[2]	Untreated	Fipronil-Permethrin	Afoxolaner
Day 7	0.5	40.8[a]	27.3[b] (33.1)	41.7[a] (0.0)
	2	36.7[a]	11.5[b] (68.6)	32.7[a] (10.9)
Day 14	0.5	45.5[a]	22.8[b] (49.8)	45.2[a] (0.7)
	2	37.2[a]	6.8[b] (81.6)	33.0[a] (11.2)
Day 21	0.5	42.2[a]	24.3[b] (42.3)	40.8[a] (3.2)
	2	38.8[a]	13.5[b] (65.2)	38.6[a] (0.6)
Day 28	0.5	37.2[a]	24.3[b] (34.5)	43.4[a] (0.0)
	2	34.7[a]	17.3[b] (50.0)	37.2[a] (0.0)

[1] Day of infestation.
[2] Time after infestation. Each dog was infested with 50 ticks on each Day at 0 h.
Repellent (on-dog) percent efficacy for either fipronil-permethrin or afoxolaner treatment of dogs relative to untreated dogs is shown in parentheses.
[a,b] Arithmetic counts within a row are significantly different $P \leq 0.05$ using both untransformed (arithmetic) and transformed (geometric) mean counts.
There were 6 animals per treatment group at each time, expect for the afoxolaner group on Days 21 and 28 for which there were 5 dogs per group.

Table 4
Total counts of attached (live and dead) *Rhipicephalus sanguineus* on dogs, and percentage anti-attachment efficacies, for animals untreated or treated with either fipronil-permethrin topically or afoxolaner orally.

Day[1]	Hour[2]	Untreated	Fipronil-Permethrin	Afoxolaner
Day 7	6	163	58 (64.4)	148 (9.2)
	12	169	22 (87.0)	124 (26.6)
	24	154	8 (94.8)	10 (93.5)
Day 14	6	164	22 (86.6)	140 (14.6)
	12	140	18 (87.1)	112 (20.0)
	24	87	7 (92.0)	8 (90.8)
Day 21	6	200	53 (73.5)	126 (37.0)
	12	171	47 (72.5)	109 (36.3)
	24	126	27 (78.6)	39 (69.0)
Day 28	6	160	62 (61.3)	103 (35.6)
	12	153	40 (73.9)	101 (34.0)
	24	116	30 (74.1)	58 (50.0)

[1] Day of infestation.
[2] Time after infestation. Each dog was infested with 50 ticks on each Day at 0 h.
Anti-attachment percent efficacy for either fipronil-permethrin or afoxolaner treatment of dogs relative to untreated dogs is shown in parentheses.
No statistical comparisons of total attached tick counts were made between treatment groups.
There were 6 animals per treatment group at each time, expect for the afoxolaner group on Days 21, 22, 28 and 29 when there were 5 dogs per group.

(Table 1) by (1) the arithmetic mean live tick counts on untreated controls at 24 h after infestation with 50 ticks per dog were from 13.3 (26.6%) to 25 (50.0%), (2) the low counts of live, free ticks at each of the 24 h counts (arithmetic mean counts ≤ 1.0 and maximum individual animal counts ≤ 2.0 across the two treatment groups for each infestation), and (3) the low counts of dead ticks on the untreated controls (arithmetic mean < 1.5, maximum ≤ 4.0 for each infestation). The viability of the flea infestations with 100 fleas per dog were confirmed by the arithmetic mean flea counts of 63.8–74.0 at 24 h after each infestation in the un-treated control group (Table 2).

The speed of kill of *R. sanguineus* (Table 1) was significantly greater for fipronil-permethrin than for afoxolaner as indicated by the consistently lower arithmetic mean, live tick counts at all times and on all days after infestation, significantly so for counts at 0.5, 2, 6 and 12 h after infestations on Days 7, 14, 21 and 28, and for the 24 h counts following the Day 28 infestation ($P \leq 0.05$). For *R. sanguineus,* acaricidal efficacy determined at 24 h was high (> 87%) and similar for the fipronil-permethrin and afoxolaner treatments after infestations on Days 7 and 14, and thereafter efficacy at 24 h gradually decreased, most notably for afoxolaner. After the Day 28 infestation, the 24 h efficacy based on arithmetic (geometric) means was 74.1% (87.0%) for fipronil-permethrin, higher than for afoxolaner which was 45.4% (59.7%).

The arithmetic mean counts of total unattached (live and dead) *R. sanguineus* remaining on the dogs provided an evaluation of repellency (Table 3). These counts, at 0.5 and 2 h after infestation on Days 7, 14, 21 and 28 were significantly lower for fipronil-permethrin than for afoxolaner ($P \leq 0.05$), and the repellent (on-dog) efficacies based on arithmetic (geometric) mean counts at 2 h ranged from 50.0% (66.1%) to 81.6% (85.1%) for fipronil-permethrin and from 0.0% (0.0%) to 11.2% (10.0%) for afoxolaner. The total counts of *R. sanguineus* (live and dead) attached on the dogs (Table 4) at 6, 12 and 24 h after infestation on Days 7, 14, 21 and 28 were lower for fipronil-permethrin than for afoxolaner, and the anti-attachment efficacies based on total counts at 12 h ranged from 72.5% to 87.1% for fipronil-permethrin and from 20.0% to 36.3% for afoxolaner. Anti-attachment efficacies based on 24 h arithmetic mean counts following the Day 7 and Day 28 infestations were 94.8 and 74.1% for fipronil-permethrin, and 93.5% and 50.0% for afoxolaner, respectively.

The speed of kill of *C. felis* (Table 2) was significantly greater for fipronil-permethrin than for afoxolaner as indicated by the significantly lower arithmetic mean, live flea counts at 0.5 h after infestation on all study days, significantly so Days 8, 15, 22 and 29 ($P \leq 0.05$). Efficacy against fleas at 0.5 h after infestation ranged from 34.1% (Day 29 infestation) to 74.7% (Day 15) for fipronil-permethrin and from 8.2% (Day 8) to 24.0% (Day 22) for afoxolaner. The *C. felis* arithmetic mean counts determined at 24 h after infestations on Days 8, 15, 22 and 29 were 0.0 or 1.0 and were similar for fipronil-permethrin and afoxolaner ($P > 0.05$), and efficacies at 24 h were ≥ 98.4% for each product for a full 4 weeks.

3.2. Safety

There were no adverse events observed that were considered to be related to treatment with either fipronil-permethrin or afoxolaner. One animal in the untreated control group developed obvious hair loss over the dorsal sacral area which was likely to have been related to the repeated parasite infestations however, it did not require any treatment intervention. One animal of afoxolaner group was diagnosed with lumbo-sacral instability and was excluded from the study on Day 20,

therefore afoxolaner group consisted of five animals form Day 20 onwards.

4. Discussion

In this study, fipronil-permethrin showed rapid repellency against new infestations of ticks as indicated 2 h after infestation by the reduced numbers of unattached ticks remaining on the dogs at 2 h, and indeed this repellent effect was apparent 30 min after infestation. This early expellency has been referred to as repellency *sensu stricto* and is likely to be an attribute of the permethrin, which has irritant and toxic effects towards the ticks, rather than of the fipronil, which has less effect on the ticks' behaviour but has strong killing potential (Halos et al., 2012). For afoxolaner, there was very little repellency, which is consistent with the mode of action of the afoxolaner and the biology of the ticks as, after infestation, ticks have to disperse, penetrate the haircoat, attach and begin feeding in order to ingest an effective dose of a systemic acaricide such as afoxolaner.

Consistent with the marked repellency effects of fipronil-permethrin on treated dogs, the numbers of ticks attached at 6, 12 and 24 h after infestation on Days 7–28 were substantially lower compared with those for untreated dogs, and compared with those for afoxolaner treated dogs, particularly at 6 and 12 h after infestation. This marked anti-attachment efficacy of fipronil-permethrin was persistent from Day 7 to Day 28 with 12 h anti-attachment efficacy of > 72% compared with < 37% for afoxolaner. In all of the treatment groups, by 6 h after infestation the majority of live ticks remaining on the animals were attached, and by 12 and 24 h there were few live unattached ticks per animal. The live tick counts for the fipronil-permethrin treatment were consistently lower than for afoxolaner up to 12 h, an effect that persisted from Day 7 to Day 28 which is likely based on the repellent effect of permethrin combined with the rapid treatment effect of fipronil. By 24 h after infestation this trend continued although the difference in counts between the active treatments reduced, presumably as ticks in the afoxolaner-treated dogs ingested increasing quantities of blood containing afoxolaner and the acaricidal action for that treatment increased. The persistence of acaricidal efficacy 24 h after infestation was readily apparent on Day 28 and was 74% (87% based on geometric means) for fipronil-permethrin compared with 45% (60%) for afoxalaner. While this difference in efficacy is most likely due to the difference in the mode of action of the active ingredients permethrin-fipronil and afoxolaner, it is noteworthy that these acaricidal efficacy values are for counts at 24 h rather than the more common 48 h counts after infestations which are often reported and typically required for regulatory purposes. The speed of kill and persistent efficacy of fipronil-permethrin reported here is comparable to that reported previously for the same product against *Ixodes ricinus*, *Dermacentor variabilis* and *R. sanguineus* (Bonneau et al., 2015a,c). The rapid expellency and anti-attachment effects are consistent with the previously reported rapid and extensive distribution of both fipronil and permethrin throughout the hair coat by 6 h after administration, and with the persistence of the actives for up to 35 days (Pellet et al., 2015). The results are also comparable to those for some other common, spot-on formulations (Bonneau et al., 2010; Baggott et al., 2011; Baker et al., 2011). Similarly, the acaricidal efficacy against ticks of afoxolaner was comparable to that reported previously for afoxolaner and other isoxazolines (Kunkle, 2014b; Geurden et al., 2016; Six et al., 2016a).

The importance of repellence, anti-attachment and rapid acaricidal efficacy to not only treat and prevent tick infestations *per se* but also to minimise the risk of tick transmission of pathogens such as *E. canis* is increasingly recognised (Dantas-Torres et al., 2012; Fourie et al., 2013a) as is their potential role in prevention of a number of other zoonotic infections, for example *Rickettsia conorii,* in which dogs and ticks have a role (Mencke et al., 2013). The use of an ectoparasiticide such as fipronil-permethrin with rapid repellency and prevention of tick attachment, in addition to its toxic or killing effect, is an important tool in the prevention and control of tick-borne diseases some of which are transmitted in less than 12 h. Products containing permethrin have also been shown to have persistent repellency effects against other vectors of canine pathogens including *Phlebotomus perniciosus* or sandflies, a vector of Leishmania species and *Culex pipiens*, the vector *of Dirofilaria immitis* and *D. repens*, canine heartworms (Franc et al., 2015a,b).

In the evaluation of flea efficacy, treatment of dogs with fipronil-permethrin provided efficacy of ≥98.4 to 100% based on arithmetic mean counts made 24 h after flea infestation (≥99.4 to 100% using geometric means) for 4 weeks after a single administration. Efficacy was similar for afoxolaner at the 24 h counts, however the immediate or knock-down efficacy was significantly greater for fipronil-permethrin and was apparent after only 0.5 h. The rapid knock-down efficacy of fipronil-permethrin in this study is similar to that reported in another study (Bonneau et al., 2015b) and is comparable to other products containing permethrin (Endris et al., 2003; Varloud and Fourie, 2015; Halos et al., 2016). The flea efficacies at 24 h after infestation for both fipronil-permethrin and afoxolaner were similar to those reported previously for these actives (Hunter et al., 2014; Kunkle et al., 2014a; Halos et al., 2016) and compares favourably with those shown by a wide variety of actives in other topical and oral products (Everett et al., 2000; Schenker et al., 2003; Franc and Bouhsira, 2009; Varloud and Fourie, 2015; Beugnet et al., 2015; Six et al., 2016b). The rapid knock-down effect of fipronil-permethrin suggests a number of secondary benefits including reduced flea bites resulting in less stimulation by flea saliva of flea allergy reactions, reduced risk of transmitting flea-borne pathogens and reduced or eliminated production of flea eggs, all of which could be evaluated in further work.

5. Conclusions

The new fipronil-permethrin combination, topical spot-on, ectoparasiticide (Effitix®) offers rapid efficacy against *R. sanguineus* and *C. felis* which persists for one month after a single administration in dogs. Afoxolaner is also effective although speed of kill is considerably slower. The rapid and sustained speed of kill of both parasites by fipronil-permethrin should contribute to the management not only of these parasites and their direct adverse effects including irritancy and allergy but also to reducing the risk of transmitting infections including some zoonoses.

Competing interests and funding

This study was financially supported by Virbac. CN is a current employee of Virbac. WN was contracted as the investigator for the study.

Authors' contributions

All authors contributed to the design of the study and interpretation of results. WN and JL managed the study, and DC and CS monitored the study. All authors read and approved the final manuscript.

Authors' information

KH is managing director, Klifovet AG.

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