Comparative Speed of Kill, Repellent (anti-feeding) and Acaricidal Efficacy of an Imidacloprid/Flumethrin Collar (Seresto®) and a Fipronil/(S)-Methoprene/Eprinomectin/Praziquantel Spot-on (Broadline®) against *Ixodes ricinus* (Linné, 1758) on Cats

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Abstract

Speed of kill, repellent (anti-feeding) and acaricidal efficacy of an imidacloprid 10 % (w/w) /flumethrin 4.5 % (w/w) collar (Seresto®, Bayer) and a spot-on formulation of fipronil 8.3 % (w/v) /(S)-methoprene 10 % (w/v) /eprinomectin 0.4 % (w/v) /praziquantel 8.3 % (w/v) (Broadline®, Merial) against artificially-induced infestations with *Ixodes ricinus* on cats, were assessed in a parallel group design, randomised, controlled study. Twenty-four cats were included and randomly allocated to treatment groups or a non-treated control group. Starting on Day (D) 7 after treatment until D28, cats were each infested with 50 *I. ricinus* at weekly intervals. Ticks were counted *in situ* on the cats at 6, 12 and 24 h and upon removal 48 h after each infestation.

Based on arithmetic means, Seresto® proved to be 100 % effective against adult *I. ricinus* at all assessment times (6, 12, 24 and 48 h after infestation) throughout the month-long study. Broadline® was 0 % to 16.7 % effective at 6 h, 26.8 % to 50.0 % effective at 12 h, while at 24 h after infestation efficacy peaked at 81.5 % on D15 declining to 31.5 % on D29. Based on the 48 h tick counts, the efficacy of Broadline® peaked at 100 % on D16 after treatment and decreased to 83.2 % by D30. The Seresto® collar provided significantly faster speed of kill and better persistent acaricidal effectiveness against *Ixodes ricinus* on cats compared to Broadline® spot-on. The additional repellent (anti-feeding) effect of Seresto® prevents parasites from taking a blood meal and thereby reduces the risk of vector-borne disease pathogen transmission.

Introduction

The ixodid tick, *Ixodes ricinus* is widespread throughout western, central and northern Europe including the northern Mediterranean countries, and is commonly associated with broadleaf temperate woodlands and forests (Estrada-Peña et al. 2004; Guglielmone et al. 2014). Small populations are present in North Africa where they are restricted mainly to the cooler and more humid regions of Tunisia, Algeria and Morocco (Walker et al. 2003). Their habitat preferences are clearly reflected at the local level by a tick survey conducted in Great Britain and Ireland, where a larger proportion of cats and dogs living in rural areas or exposed to woodland or moorland were infested with *I. ricinus* than those living in urban or suburban dwellings, or exposed to urban parks or farm pastures (Ogden et al. 2000). An increase in its distribution range because of milder winter temperatures associated with climate change is a distinct probability in northern Europe (Lindgren et al. 2000).

All development stages of *I. ricinus* infest a wide range of mammals, both wild and domestic, and the immature stages may also infest birds and reptiles (Estrada-Peña et al. 2004; Guglielmone et al. 2014). This wide range of potential hosts is especially important given the vector potential of *I. ricinus*, which can transmit pathogens of severe animal and human diseases e.g. *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*. This vector potential links *I. ricinus* ticks up with many other ectoparasites on dogs and cats (e.g. several other tick species and fleas) which have come into particular focus during the recent decade due to their important role as vectors of already established as well as emerging vector borne diseases.

Ectoparasite control on our companion animals, especially flea and tick control, is for this reason more than a pure hygienic factor; it is moreover essential for safeguarding their health and the health of their owners. While for flea control on dogs and cats as well as tick control on dogs a large

number of authorised products containing different active ingredients are available, the choice for tick control on cats is limited. For a long time fipronil containing products were the main available option for the treatment of ticks on cats, as the majority of acaricidal actives used on dogs – especially those offering the additional benefit of repellency (e.g. permethrin) or expellency (e.g. amitraz) (Halos et al. 2011) – are contraindicated for the use in cats. Recently introduced slow-release matrix collars containing imidacloprid 10 % (w/w) /flumethrin 4.5 % (w/w) (Seresto®, Bayer) have proven to be highly effective for up to 8 months against infestations of ixodid ticks and fleas on cats (Stanneck et al. 2012a, Stanneck et al. 2012b). Its acaricidal active flumethrin is the first on the market to provide acaricidal (killing) and repellent (anti-feeding) efficacy for the treatment and prevention of tick infestations on cats, thus preventing repelled parasites from taking a blood meal and thereby indirectly aids in the reduction of the risk of vector-borne disease transmission, as demonstrated for the prevention of transmission of *Cytauxzoon felis* by *Amblyomma americanum* ticks (Reichard et al. 2013). In addition, an effective repellent or speed of kill effect against ticks (in particular *I. ricinus*) is also evident for 34 weeks after application of the collars. A recently introduced combination of fipronil 8.3 % (w/v) / (S)-methoprene 10 % (w/v) / eprinomectin 0.4 % (w/v) / praziquantel 8.3 % (w/v) in a spot-on formulation (Broadline®, Merial) for cats uses again fipronil as the acaricidal component of its ecto- and endoparasite combination and has proven to be effective against laboratory infestations of *I. ricinus* for 3 to 5 weeks after treatment (Tielemans et al. 2014b).

In this communication we report on the comparative speed of kill, repellent (anti-feeding) and acaricidal efficacy of the imidicloprid/flumethrin collars and the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on against *I. ricinus* on domestic cats.

Material and methods

Ethical considerations

This study was conducted in the spirit of VICH GL9 guideline on Good Clinical Practice, and all procedures were in compliance with South African Animal Welfare Act Regulations ‘The care and use of animals for scientific purposes’. All the cats were examined by a veterinarian before their inclusion in the study and were observed for general health at least once daily during the course of the investigation, and the treated groups also at hourly intervals for 4 hours after treatment.

Animals

Thirty-two cats were initially enrolled in the study and thereafter reduced to 24 animals based on pre-study attached live female tick counts. The cats selected were of mixed breed, mostly short-haired (hair length 15.5 mm to 28.5 mm), of both sexes, older than 6 months, weighed between 1.71 kg and 4.75 kg and had not been treated with a long acting topical or systemic acaricide during the 12 weeks preceding the commencement of the study.

The study followed a randomised block design and the cats were initially divided into two body weight categories, namely 12 whose body weights <2.50 kg, and 12 whose body weights ≥2.50 kg. Within each body weight category, the animals were ranked within sex in descending order of individual pre-treatment live attached *I. ricinus* tick counts. The cats within each category were subsequently blocked into four blocks of three cats each, and within blocks randomly allocated to the three groups using random numbers generated in Microsoft Excel, and sorting these numbers in ascending order. The three groups each consisted of three males and five female cats and thus with 8 animals per group exceeded the requirements of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.), “Guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick

infestations on dogs and cats” of at least six animals per group (Marchiondo et al. 2013).

All cats were dewormed and did not harbour ticks or fleas at the commencement of the study. The animals were kept individually in cages in a controlled environment within an indoor animal unit and no contact between cats was possible. They were fed an age appropriate commercial cat diet and fresh, clean water was provided. The identification, sex, group number and ration of each cat were specified on the outside of each cage.

Tick infestation

A laboratory-bred strain of *I. ricinus* of European origin was used for all infestations. Immature ticks were fed on rabbits, and the adults used for infestation, were unfed, at least one week old and of a balanced sex ratio (50 % female: 50 % male). Each cat was artificially infested with 50 ticks (50 % male/50 % female) on Day 7 after treatment with the investigational products, and at weekly intervals thereafter until Day 28 (Table 1). The time of infestation was recorded for each animal to ensure that the *in situ* counting of ticks at 6 h (± 5 min), 12 h (± 30 minutes) and 24 h (± 1 h) and their removal at 48 h (± 2 h) after infestation were accomplished as closely as possible to the specified target times. During *in situ* counts ticks were found by direct observation following parting of the hair and by palpation with the tips of the fingers. During removal counts the same procedure was followed but ticks were removed upon counting and the cats were also combed to ensure that all ticks were counted and removed.

Treatment

Commercially available imidacloprid/flumethrin collars (Seresto®, Bayer) for cats were fitted to the designated group of eight cats on Day 0: According to administration instructions the collar was adjusted around the animal’s neck allowing for a space of two fingers between the collar and neck. The tip of the collar was pulled through the loop and any length in excess of 2 cm was cut off.

Table 1 Study design to determine acaricidal, repellent and speed of kill efficacy of Seresto® and Broadline® against adult *Ixodes ricinus* on cats

Day	Procedure
- 7 to - 1	Acclimatization to cage environment
- 7	Tick infestation
- 5	Tick counts
- 2	Ranking on Day -5 tick counts, allocation to three treatment groups
0	Seresto® collars fitted and Broadline® spot-on applied
7	Tick infestation and <i>in situ</i> tick counts 6 h and 12 h after infestation
8	<i>In situ</i> tick counts 24 h after infestation
9	Tick counts and tick removal 48 h after infestation
14, 15 and 16	Repetition of activities performed on Days 7, 8 and 9
21, 22 and 23	Repetition of activities performed on Days 7, 8 and 9
28, 29 and 30	Repetition of activities performed on Days 7, 8 and 9

Table 2 Status of adult *Ixodes ricinus* counted and removed from cats 48 hours after weekly infestation from Day 7 to 28 after fitting Seresto® collars or applying Broadline® topically

Survival status	Attachment status	Interpretation
Live	Free or attached ^a	Acaricidal effect not demonstrated
Dead	Free or attached	Acaricidal effect demonstrated

^a If justified, only live attached tick counts may be used to assess efficacy for systematically acting acaricides.

The commercially available spot-on formulation of fipronil/(S)-methoprene/eprinomectin/praziquantel (Broadline®, Merial) was administered using the single-dose applicators. Cats weighing 0.8 kg to <2.5 kg were treated with the contents of the 0.3 ml applicator and those weighing 2.5 kg to <7.5 kg received the contents of a 0.9 ml applicator. Treatment was administered on Day 0

according to administration instructions by parting the hair on the midline of the neck, between the base of the skull and the shoulder blades and delivering the contents of the syringe directly onto the skin in a single spot. The cats were restrained for approximately one minute following administration to prevent any possible run-off, but none was observed.

After treatment the cats in both groups were observed hourly for 4 hours for any adverse symptoms that could be related to treatment. The cats in the control group remained untreated. A tabulated overview of this study is given in Table 1.

Tick counts

All ticks counted and removed were categorised as live or dead and free or attached (Table 2). Live free or attached ticks were considered treatment failures (Marchiondo et al. 2013).

The primary assessment norm was the number of ticks counted on the untreated control and the two treated groups of cats at the various assessment times and days, with efficacy calculations based on arithmetic means. Since only the females of *I. ricinus* attached and actively fed on the cats the statistical analyses were performed taking only the numbers of female ticks into account. Efficacy of Seresto® collars and Broadline® spot-on against adult *I. ricinus* at 6, 12, 24 (for assessment of speed of kill and repellent efficacy) and 48 h (for assessment of acaricidal efficacy) after infestation was calculated as follows:

$$\text{Efficacy (\%)} = 100 \times (M_c - M_t) / M_c, \text{ where}$$

M_c = Arithmetic mean number of live ticks on cats in the untreated control group at a specific time point.
 M_t = Arithmetic mean number of live ticks on cats in the respective treated groups at a specific time point.

The tick counts of the two treated groups were compared using an ANOVA with a treatment effect after a logarithmic transformation on female tick count (count + 1) data. SAS Version 9.3 TS Level 1M2 was used for all the statistical analyses.

Table 3 Acaricidal efficacy of Seresto® and Broadline® against adult *Ixodes ricinus* on cats based on arithmetic mean female tick counts at 6, 12, 24 and 48 hours after weekly infestation

Study day	Count time-point	Arithmetic mean numbers of ticks (range)			Efficacy (%) ¹		Seresto vs Broadline
Infestation	Tick count ^{3,4}	Control	Seresto	Broadline	Seresto	Broadline	p-value ²
D7	6h (D7)	6.9 (3 – 12)	0.0	7.4 (1-14)	100	0.0	<.0001
	12h (D7)	9.4 (4-15)	0.0	6.0 (0-12)	100	36.0	0.0015
	24h (D8)	9.3 (5-15)	0.0	2.6 (0-7)	100	71.6	0.0868
	48h (D9)	10.0 (6-17)	0.0	0.5 (0-1)	100	95.0	0.6824
D14	6h (D14)	12.0 (8-16)	0.0	10.0 (5-16)	100	16.7	<.0001
	12h (D14)	9.5 (6-13)	0.0	4.8 (1-14)	100	50.0	0.0040
	24h (D15)	10.1 (7-14)	0.0	1.9 (0-3)	100	81.5	0.0492
	48h (D16)	9.8 (6-14)	0.0	0.0 (0)	100	100.0	1.0000
D21	6h (D21)	7.8 (2-15)	0.0	6.6 (3-11)	100	14.5	0.0001
	12h (D21)	6.6 (3-12)	0.0	4.6 (2-7)	100	30.2	0.0005
	24h (D22)	7.0 (3-12)	0.0	1.5 (0-4)	100	78.6	0.1576
	48h (D23)	7.6 (4-14)	0.0	0.3 (0-2)	100	96.7	0.8222
D28	6h (D28)	13.0 (10-20)	0.0	11.5 (7-19)	100	11.5	<.0001
	12h (D28)	12.1 (8-17)	0.0	8.9 (5-15)	100	26.8	<.0001
	24h (D29)	11.5 (8-18)	0.0	7.9 (4-14)	100	31.5	<.0001
	48h (D30)	11.9 (7-19)	0.0	2.0 (0-7)	100	83.2	0.1374

¹ Percentage efficacy = 100[(C – T)/C], where C and T are the arithmetic mean female tick counts

² p-value: One-way ANOVA with a treatment effect; ³ h = hours; ⁴ D = study day

Results

Both treatments were well tolerated by the cats and no adverse reactions were observed.

The efficacies of the imidacloprid/flumethrin collars and the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on at 6, 12, 24 and 48 h after infestation on Days 7, 14, 21 and 28 are summarised in [Table 3](#).

The mean number of female ticks counted upon removal at 48h from the untreated control cats varied between 7.6 and 11.9, corresponding to ≥30% attachment rate of female ticks used for infestation and representing a vigorous challenge.

Acaricidal efficacy based on 48 hour tick counts

There was a significant difference ($p < 0.05$) in the arithmetic mean number of ticks recovered between the control group of cats and the two treated groups on all assessment days. No significant difference ($p > 0.05$) in arithmetic mean tick counts was observed at any of the 48 hour assessment time points between the two treated groups. Acaricidal efficacy in the group of cats fitted with the imidacloprid/flumethrin collars was 100% throughout the study, while efficacy in the group of cats treated with the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on peaked at 100% on Day 16 but had decreased to 83.2% by Day 30.

Fig. 1 The efficacy of Seresto® and Broadline® against adult *Ixodes ricinus* on cats 6, 12 and 24 h after infestation at weekly intervals from Day 7 to Day 28 after treatment

Speed of kill and repellent efficacies based on *in situ* tick counts at 6, 12 and 24 hours

The speed of kill efficacies of the imidacloprid/flumethrin collars and fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on are graphically illustrated in Figure 1.

There was a significant ($p < 0.05$) difference in the arithmetic mean number of ticks between the control group and the imidacloprid/flumethrin collar treated group at all time points and assessment days. No live ticks were found at any time during the *in situ* examinations of the cats fitted with the imidacloprid/flumethrin collars. Efficacy against adult *I. ricinus* was thus 100% at 6, 12 and 24 h throughout the 30 days of the study.

For the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on the difference between the arithmetic mean number of ticks on the control group of cats and the group treated with the spot-on was significant ($p < 0.05$) only at the 24 h time point on all

assessment days, when efficacy peaked at 81.5% on Day 15, decreasing thereafter to 31.5% by Day 29. The differences at the 12 h time point between the tick counts on the control group of cats and the spot-on treated cats were significant ($p < 0.05$) only on Days 14 and 28 when efficacies of 50% and 30.2% were measured. At 6 h post-infestation no significant differences ($p > 0.05$) were evident for any of the assessment days between the tick counts of the control and spot-on treated groups of cats and efficacies varied between 0% on Day 7 and 16.7% on Day 14. For the 6, 12 and 24 h time points the arithmetic mean tick counts on the collared group of cats were at all times significantly lower ($p < 0.05$) than those on the cats treated with the spot-on except for the 24 hour points on Days 8 and 22. The efficacy of the spot-on increased as the exposure time of the ticks to the treated cats increased, with efficacy for each time point peaking at the D14 assessment day and declining again afterwards.

Discussion

In three previous studies the imidacloprid/flumethrin collars proved to be 100 % effective against laboratory-induced infestations of adult *I. ricinus* on domestic cats on Day 7 post-treatment and thereafter against re-infestation at 4-weekly intervals from Day 28 until 8 months later (Stanneck et al. 2012a). Speed of kill efficacy 6 h after infestation was also 100 % over the same period of time in two of the studies in which this attribute was tested (Stanneck et al. 2012a). The speed of kill as well as repellent and acaricidal efficacy now recorded against weekly infestations of adult *I. ricinus* over a period of 4 weeks, are in agreement with those of the previous studies, as no ticks, dead or alive, were detected on the treated cats at any of the measured time points and assessment days.

In three published studies on the *I. ricinus* efficacy of the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on tick counts were performed at 48 h post infestation and results are given as geometric mean efficacy values only. In these studies the 48h geometric mean efficacy against *I. ricinus* increased to >98 % against subsequent weekly infestations of ticks until 30 days after treatment in two of the studies and until 25 days in the third, decreasing to 88.7 % at 30 days in the latter study (Tielemans et al. 2014b). By Day 37 efficacy was 97.6 % in one of the studies, 93.1 % in another and 85.1 % in the third. The length of one of the studies was increased by a week and an efficacy of 85.4 % was recorded on Day 44 in this study. The arithmetic mean results obtained at 48 h after infestation in the current study are somewhat in accordance with those of the previous studies, with arithmetic mean efficacy peaking at 100 % on Day 16 and afterwards decreased to 83.2 % by Day 30. This is also in accordance to the European Public Assessment Report of Broadline®, where the results of five dose confirmation studies involving *I. ricinus* showed variable durations of efficacy of 9 to 37 days with the pooled data showing efficacy >90 % against *I. ricinus* until Day 14 only,

however, 98 % efficacy was achieved after 3 weeks in 3 out of the 5 studies.

No studies were so far available assessing speed of kill and repellent efficacies of the fipronil/(S)-methoprene/eprinomectin/praziquantel spot-on. None of the arithmetic efficacy data obtained at *in situ* tick counts at 6, 12 and 24 hours at the different time points of the current study reached the 90 % efficacy threshold.

Re-treatment at 4 to 5 week intervals will be necessary to achieve continuous tick control, particularly during periods of high tick challenge, making owner compliance an important factor of successful tick control.

The Seresto® collar provided significantly faster speed of kill and better persistent acaricidal effectiveness against *I. ricinus* on cats compared to Broadline® spot-on. The additional repellent (anti-feeding) effect of Seresto® prevents parasites from taking a blood meal and thereby reduces the risk of vector-borne disease pathogen transmission.

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Ethical Standards:

The study was performed in compliance with South African Animal Welfare Act Regulations 'The care and use of animals for scientific purposes'

Conflict of interests

The study reported here was funded by Bayer Animal Health of whom Katrin Deuster and Bettina Schunack are employees. The other authors are employees of ClinVet International, an independent Contract Development Organisation, contracted to manage the conduct of the study, while Ivan

Horak is also an Emeritus Professor in the Faculty of Veterinary Science, University of Pretoria. All authors voluntarily publish this article and have no personal interest in this study other than publishing the scientific findings that they have been involved in via planning, setting-up, conducting and compiling and analysing the results.

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