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Original Article

Repellency Effect of an Imidacloprid/Flumethrin (Seresto®) Controlled Release Polymer Matrix Collar against the Australian Paralysis Tick (*Ixodes holocyclus*) in Dogs

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Abstract

Repellency is a highly desirable attribute for an acaricide that protects dogs against the Australian paralysis tick (*Ixodes holocyclus*). A bite from a single tick of this species can be fatal and carries the risk of transmission of vector-borne diseases. A randomised, controlled study was conducted to confirm the repellency *sensu stricto* and *sensu lato* (expellency) of a 10% imidacloprid/4.5% flumethrin collar (Seresto®, Bayer) on dogs, against artificial infestations of *I. holocyclus*. Based on a novel protocol, dogs treated with Seresto®, or a placebo collar, were sedated and placed in infestation chambers for 1 h at varying time points up to 6 months after treatment. Ticks were released alongside the dogs, and allowed to approach and/or attach. Treating dogs with a Seresto® collar resulted in significantly ($p < 0.001$) more residual ticks being found in the infestation chambers (repellency *sensu stricto*) compared to the placebo treated dogs for the 168-day duration of the study (range 67.5% to 90.2%). Repellency *sensu lato* (expellency) was reflected in significantly ($p < 0.002$) fewer ticks being found attached to treated dogs throughout the study. Comparative decrease in ticks attached exceeded 95% for 84 days, when assessed after 6 h. When assessed after 24 h, there was a >95% comparative decrease in ticks attached until Day 84, >90% on Days 112 and 140 then >95% to Day 168. Efficacy (killing effect) of the Seresto® collar based on total live tick counts was >95% 6 h after tick challenge throughout the 6 month study period. This study demonstrated the excellent repellency effect of the Seresto® collar against *I. holocyclus* in dogs.

Introduction

As it feeds, the adult, female Australian paralysis tick (*Ixodes holocyclus*) injects a potent neurotoxin (holocyclotoxin) into its host (Fig. 1). A single tick bite is capable of causing paralysis and death in small mammals, and also carries the risk of transmission of vector-borne diseases (VBD) (Gofton et al. 2015). Other potential effects include blood loss, immunosuppression, secondary infection at the bite site and localised dermatitis (Greay et al. 2016). Effective tick control is therefore essential for dogs and cats, particularly those living along the eastern coast of Australia where *I. holocyclus* is endemic. Acaricides that show a repellent effect in addition to killing capacity are highly preferable against this ectoparasite.

Fig. 1 Adult female, partially engorged Australian paralysis tick (*Ixodes holocyclus*)

Ixodid ticks (such as *I. holocyclus*) acquire their hosts by climbing vegetation and carrying out questing behaviour. They detect the host by a range of stimuli including vibrations, CO_2 concentrations and host odour (Padula 2015). Due to this complex parasite-host interaction and vector role of ticks, the total benefit to the host of an acaricide cannot be completely described by a single efficacy value (Marchiondo et al. 2013).

Acaricides are effective in controlling ticks in three main ways, either solely, or in combination. Repellency *sensu stricto* refers to an irritant effect that causes the tick to move away from the treated animal, leading to avoidance, or to fall off the animal soon after contact with the hair coat (Halos et al. 2012). This is true repellency, as ticks are removed/killed before they can bite. Repellency *sensu lato*, or 'expellency' refers to the combined effect of disruption of attachment and causing the tick to fall off the host within 24 h (Halos et al. 2012). The term 'killing effect' is used to describe the ability of an acaricide to induce death of the tick. This term can also be interchanged with overall efficacy of an acaricide.

Acaricides with repellent properties *sensu stricto* have obvious benefits over acaricides that act primarily by expellency and/or killing effect as they prevent tick bites, thus preventing VBD and irritation effects, as well as avoiding intoxication (Marchiondo et al. 2013). In the case of systemically active acaricides such as chewable and tablet formulations, ticks must bite their host in order to be affected. Ticks that attach and are killed may remain attached, which may lead to uncertainty by pet owners and veterinarians, as doubt remains around the risk of intoxication.

The Seresto® collar (Bayer, 10% imidacloprid, 4.5% flumethrin), is a polymer matrix collar for dogs and cats that allows a continuous and prolonged release of the active ingredients imidacloprid, a chloronicotinyl compound, and flumethrin, an α-cyanopyrethroid, onto the skin surface and hair coat of treated animals (Stanneck et al. 2012a). It has been shown to achieve long-term persistent efficacy of 8 months against fleas (*Ctenocephalides felis felis*) (Stanneck et al. 2012b, 2012c) and 4 months against *I. holocyclus* on dogs (Smith et al. 2013). The Seresto® collar has also been shown to be

highly effective in the prevention of transmission of VBD from ticks to dogs (Dantas-Torres et al. 2013, Stanneck et al. 2013).

The current study was conducted to determine the repellency *sensu stricto* and *sensu lato* (expellency) of the Seresto® collar, over a 6 month period, against *I. holocyclus* in dogs. In addition, overall efficacy (killing effect) was assessed.

Materials and methods

Study design

A randomised, placebo-controlled, unblinded pen study was conducted at a contract research facility to determine the repellency *sensu stricto* and *sensu lato* (expellency) of a 10% imidacloprid/4.5% flumethrin collar (Seresto®, Bayer Animal Health) against *I. holocyclus* in dogs, from application of the collar on Day 0 until Day 173 (6 month duration). The experimental design is outlined in Table 1.

Study animals

Study animals were clinically healthy, mixed-sex foxhound or foxhound-cross dogs (n = 16), 2.2–7.8 years of age, weighing 27.6–39.5 kg. No ectoparasiticides were applied to the dogs for 90 days prior to study commencement. Throughout the study dogs were treated with a broad-spectrum anthelmintic every 4–6 weeks, or as required. Dogs were fed a maintenance quantity of complete, dry dog

Table 1 Overview of experimental design and activities

Days	Activity
-10	Veterinary examination, all dogs (n=16).
-7 to -3	Tick carrying capacity (TCC) test. Dogs ranked according to TCC, weight, age and gender. Allocation of dogs to treatment Group 1 (placebo collars n=8) or Group 2 (Seresto® collars, n=8), and Cohorts A and B.
0	Weigh Cohort A dogs, treat Cohort A dogs.
2	Weigh Cohort B dogs, treat Cohort B dogs.
6–7	Fast Cohort A dogs overnight. Weigh Cohort A dogs, sedate, tick challenge, count ticks remaining in infestation chambers (+1 h) count ticks on dogs (+6 h).
8	Count ticks on Cohort A dogs and remove (+24 h). Fast Cohort B dogs overnight.
9	Weigh Cohort B dogs, sedate, tick challenge, count ticks remaining in infestation chambers (+1 h) count ticks on dogs (+6 h).
10	72 hour tick search Cohort A dogs (remove any remaining ticks). Count ticks on Cohort B dogs and remove (+24 h).
12	72 hour tick search Cohort B dogs (remove any remaining ticks).
27–33	Repetition of activities performed on Days 6–12.
42	Infest Group 1 dogs (untreated) with 10 ticks each to maintain hyper-immunity.
55–61	Repetition of activities performed on Days 6–12.
70	Repetition of activities performed on Day 42.
83–89	Repetition of activities performed on Days 6–12.
98	Repetition of activities performed on Day 42.
111–117	Repetition of activities performed on Days 6–12.
139–145	Repetition of activities performed on Days 6–12.
154	Repetition of activities performed on Day 42.
167–173	Repetition of activities performed on Days 6–12.
173	End of study.

food once or twice daily and had ad libitum access to clean water.

All study dogs were hyperimmunised against *I. holocyclus* holocyclotoxin by exposing them to gradually increasing numbers of unfed adult female ticks prior to the start of the study. Tick carrying capacity (TCC) was determined for each dog between Day -7 to Day -3 prior to treatment. TCC was used to rank, block and assign each dog to treatment Group 1 (placebo collar n=8) or treatment Group 2 (Seresto® collar n=8). Treatment groups were equivalent in terms of weight, age, gender and TCC. Parametric One-Way Analysis of Variance was used to compare groups prior to treatment, using a fixed-effects linear model and the statistical package Spotfire S+ Version 8.2, Tibco Software Inc. 2010.

Within each group, dogs were allocated to Cohort A (n=4) or Cohort B (n=4), such that each cohort had a similar TCC and range of TCC's within the group. Allocation to cohorts was to allow time for all scheduled activities to be conducted within the timeframe required, with Cohort B activities conducted 48 h after Cohort A activities. One additional hyperimmune dog was enrolled in the study on Day 50 (in the placebo Group 1), to replace a dog that had escaped from an exercise run. It was determined that this substitution did not affect the statistical analysis of the study.

At all times, the Seresto® and placebo-treated dogs were kept separate and study personnel followed appropriate measures to prevent transfer of active ingredients or ticks between dogs. Study animals were housed in indoor/outdoor pens measuring 1.5 m x 6 m for the duration of the study. Access to outdoor areas allowed for confirmation of the long term photo-stability of the Seresto® collar (Marchiondo et al. 2013). On days when they were not tick challenged, dogs of the same group were housed in socially compatible pairs and allowed access to an adjacent pen to enhance animal welfare. Dogs were exercised within treatment groups with no contact between treatment groups permitted. Interior pens were air-conditioned, and dogs had free access to the interior and exterior pens.

Treatment and tick infestation

Group 2 dogs were treated with commercially available imidacloprid/flumethrin collars for dogs over 8 kg (Seresto®, Bayer). Group 1 dogs were treated with either a placebo collar (Seresto® collar with no active ingredients) or a commercially available, non-acaricidal plastic dog collar of similar size and weight to the Seresto® collar.

Cohort A dogs were weighed on Day 0, Cohort B dogs on Day 2, and either Seresto® or placebo collars applied. Collars were fitted to the neck of each dog, leaving a gap of approximately 2.5 cm between the collar and the neck. The excess lengths of the collars were fitted through collar loops and single mattress sutures were placed to secure the free ends to the underlying collars. The collars were retained for the duration of the study, except in the case of collar loss, in which case they were re-attached or replaced as soon as the loss was observed. All dogs were examined three times daily for any signs of ill health.

Unfed adult female *I. holocyclus* were collected from at least three different localities in the Northern Rivers area of New South Wales. The ticks were maintained in the dark, at optimal conditions of temperature and humidity (Goodrich et al. 1978) prior to use. Tick challenges commenced on Day 7 for Cohort A and Day 9 for Cohort B dogs using a method modified from Marchiondo et al. (2013). All dogs were fasted the night prior to tick challenges.

On the day of tick challenges, dogs were weighed and dose volumes of the sedative, medetomidine hydrochloride (Domitor®, Zoetis) were calculated. Dogs were sedated by intravenous or intramuscular administration then placed in large plastic, uncovered 'infestation chambers' within the dog's

normal pens, and monitored closely. Dogs in Group 1 (untreated) were always challenged first before Group 2 (treated) animals. The chambers had double-sided tape placed around the top perimeter of the walls to prevent the ticks from escaping.

Temperature inside the pens was maintained at a minimum of 18°C during the tick challenge process and until all ticks had been removed. Each dog was placed centrally, in lateral or sternal recumbency in the infestation chamber and 50 ticks were released alongside the dog, within 20 cm of the dog; 10 either side of the head, 10 either side of the forequarters, and 5 either side of the hindquarters. Dogs remained in the chambers for 1 h ± 5 min, before being removed and returned to their pens. Care was taken not to dislodge any ticks on the dogs in the moving process. Any ticks found on clothing, hands or arms of the animal technicians whilst they were in the pens, that had been picked up from a particular dog were placed back on that dog immediately after it had been moved. Any ticks that were found by technicians on their person after leaving dogs pens were disposed of. Ticks remaining in the infestation chamber were counted, discarded and the chamber removed. Subsequent tick challenges were performed on days 28, 56, 84, 112, 140 and 168 (Cohort A) or days 30, 58, 86, 114 and 170 (Cohort B).

For both cohorts, ticks that remained on the dogs were counted, and classified, at 6 h ± 30 min and 24 h ± 1h post-challenge. Ticks were assessed, removed and discarded at the 24 h count. Dogs were housed individually during tick challenges until after the 24 h count.

Dogs were examined again 3 days (approximately 72 h) post-challenge to locate and remove any remaining ticks. This examination was a safety precaution to minimise the risk of potential tick paralysis from any previously undiscovered paralysis ticks.

Tick counts

Dogs were examined for ticks using a systematic method of digital palpation and visual inspection. Ticks were classified as shown in Table 2. Classification was a subjective process undertaken by experienced tick assessors. Marchiondo et al. (2013) defined an engorged tick as "an adult female ixodid ('hard tick') that has taken a single large blood meal to produce a conspicuous filling of the alloscutum". Ticks remaining in the infestation chambers were counted and classified after the dogs were removed following the 1 h tick challenges. These counts were used to calculate the repellency effect *sensu stricto* (Marchiondo et al. 2013).

Table 2 Classification of ticks following tick challenges

Category	Viability	Attachment Status	Notation[a]
1	Live	Free. unengorged, engorging or engorged	LFU / LFe / LFE
2	Live	Attached, un-engorged	LAU
3	Live	Attached, engorging / engorged[b]	LAe / LAE
4	Dead	Free. unengorged, engorging or engorged	DFU / DFe / DFE
5	Dead	Attached, un-engorged	DAU
6	Dead	Attached, engorging	DAe
7	Dead	Attached, engorged[b]	DAE

[a] Adapted from Marchiondo et al. (2007). L = Live; F = Free (all unattached ticks, live and moving through the coat, or dead and sitting in the hair); U = Un-engorged (no swelling); e = engorging (having a conspicuous swelling of the alloscutum, may be wider and longer than un-engorged ticks and contain blood); E = Engorged (12–15 mm long and 8–10 mm wide, with a turgid appearance); D = Dead

[b] In general, engorged *I. holocyclus* ticks are not seen until 6 days post-challenge.

The total attached tick (ToA) count consisted of all attached live and dead ticks (LAU+LAe+DAU+DAe) found on the dogs at 6 and 24 h post-challenge. The repellency effect *sensu lato* (or expellency) was calculated by comparison of treated and untreated group mean ToA at 6 and 24 h (Marchiondo et al. 2013).

The total live tick (ToL) count was also performed at 6 and 24 h post-challenge. This count included all live ticks attached or unattached (LFU/LFe/LFE, LAU/LAe /LAE). This count was included in the study to indicate the killing effect, or overall efficacy, of the Seresto® collar.

Data analysis

The number of dogs enrolled in the trial was based on the recommendations of Marchiondo et al. (2013). In order to determine the repellency effect, group arithmetic mean tick counts for residual ticks in the infestation chambers (*sensu stricto*), and group geometric mean tick counts of ToA (LAU+LAe+DAU+DAe) at the post-challenge counts at 6 and 24 h (*sensu lato* or expellency), were calculated from the raw data (Halos et al. 2012). Tick counts for Cohorts A and B were combined (within treatment groups) at each time point for calculations and analysis.

$$\text{Repellency effect } \textit{sensu stricto}\ (\%) = \frac{\text{Mean residual tick Seresto® Count} - \text{Mean Placebo Count}}{\text{Mean Seresto® Count}}$$

$$\text{Repellency effect } \textit{sensu lato}\ (\%) = \frac{\text{Mean ToA tick Placebo Count} - \text{Mean Seresto® Count}}{\text{Mean Placebo Count}}$$

Overall efficacy was calculated using geometric mean ToL counts at 6 and 24 h after each tick challenge:

$$\text{ToL} = \text{LFU} + \text{LFe} + \text{LFE} + \text{LAU} + \text{LAe} + \text{LAE}$$

Group mean tick counts were compared at a family-wise significance level of $p<0.05$ using Tukey's multiple comparison test. Residual and ToA tick counts appeared to be approximately normally distributed within the overall group of selected dogs, with similar median and mean values. When standard deviations were expressed as a percentage of the group mean (co-efficient of variation) they were relatively small, indicating relatively low variability in the data.

Homogeneity of variances for residual and ToA tick counts post infestation were tested using Levene's Test (calculated using Statistix 10.0, Analytical Software 2013), to determine the suitability of parametric tests (One Way Analysis of Variance) for comparison of group means. Residual tick counts were broadly acceptable to be used untransformed, however ToA counts required log-transformation for statistical comparisons. No additional statistics were performed on the ToL counts.

Results

Treating dogs with a Seresto® collar resulted in significantly ($p<0.001$) more residual ticks being found in the infestation chambers for the 168-day duration of the study, compared to the placebo group. Repellency *sensu stricto* varied between 67.5 and 90.2%, with an overall average of 79% (Table 3).

Repellency *sensu lato* was demonstrated by significantly ($p<0.002$) fewer ticks being found attached to treated dogs at either 6 or 24 h post-challenge throughout the study. The treatment effect exceeded 95% for 84 days when assessed after 6 h. When assessed after 24 h, there was a >95% effect through until Day 84, >90% on Days 112 and 140 then >95% on Day 168 (Table 4).

No live ticks were found attached to any treated dog until Day 84. Efficacy, calculated using Geometric mean ToL counts was >95% from 6 h after tick challenge throughout the study (Table 5).

Table 3 Repellency effect *sensu stricto* of Seresto® collars against *I. holocyclus* in dogs based on group arithmetic mean residual tick counts from infestation chambers 1 h post-challenge.

Day of Infestation post treatment	Arithmetic mean no. ticks remaining in the infestation chamber		Repellency *sensu stricto* (%)	Seresto® vs Placebo p-value
	Placebo	SERESTO		
7	14.8	46.5	68.3	< 0.001
28	11.0	44.4	75.2	< 0.001
56	6.3	44.8	86.0	< 0.001
84	6.8	43.9	84.6	< 0.001
112	3.9	39.4	90.2	< 0.001
140	12.9	39.6	67.5	< 0.001
168	6.8	39.7	83.0	< 0.001

Table 4 Repellency *sensu lato* of Seresto® collars against *I. holocyclus* in dogs, based on group geometric mean total attached tick (ToA) counts at 6 and 24 h post-challenge.

Day of Infestation post treatment	Time point post infestation (h)	Geometric Mean ToA tick counts		Repellency *sensu lato* (%)	Seresto® vs Placebo p-value
		Placebo	SERESTO		
7	6	19.5	0.6	97.1	< 0.001
	24	21.4	0.4	98.3	< 0.001
28	6	19.7	0.5	97.5	< 0.001
	24	22.4	0.5	97.8	< 0.001
56	6	21.2	0.2	98.9	< 0.001
	24	26.4	0.2	99.3	< 0.001
84	6	24.6	0.5	98.0	< 0.001
	24	29.6	0.4	98.8	< 0.001
112	6	27.3	2.3	91.6	< 0.001
	24	30.5	2.6	91.4	< 0.001
140	6	23.3	3.1	86.7	< 0.002
	24	26.0	2.5	90.3	< 0.001
168	6	31.2	1.0	96.7	< 0.001
	24	32.2	1.2	96.1	< 0.001

Collars were generally well tolerated by the dogs. One adverse reaction was reported at Day 126 in one dog wearing a placebo collar. An area of superficial dermatitis developed on the dogs' ventral neck, adjacent to and under the collar. This event was possibly attributed to the placebo treatment. The dermatitis resolved following a 14 day course of topical and systemic antimicrobial treatment (Rilexine® tablets, Virbac; Neocort® cream, Ilium). This adverse event was determined to have no impact on the statistical results of the study.

Discussion

Measurement of repellency *sensu stricto* following the method outlined by Marchiondo et al. (2013) is reported for the first time, to the knowledge of the authors, in this study. The Seresto® collar was proven to have an excellent repellent effect *sensu stricto* against *I. holocyclus* in dogs, ranging between 67.5 and 90.2%, with an overall average of 79%, measured over a 6 month period.

Table 5 Efficacy of Seresto® (killing effect) against *I. holocyclus* in dogs based on group geometric mean total live tick (ToL) counts at 6 and 24 h post-challenge.

Day of Infestation post treatment	Time point post infestation (h)	Geometric mean no. ToL ticks		Efficacy (killing effect) %
		Placebo	Seresto®	
7	6	19.1	0.0	100.0
	24	19.9	0.0	100.0
28	6	19.1	0.0	100.0
	24	22.0	0.0	100.0
56	6	22.0	0.0	100.0
	24	25.6	0.0	100.0
84	6	24.2	0.1	99.4
	24	27.8	0.1	99.5
112	6	27.4	1.1	95.9
	24	29.6	1.0	96.8
140	6	23.1	0.9	96.3
	24	25.8	0.5	98.2
168	6	32.3	0.5	98.6
	24	31.7	0.2	99.4

Several studies have verified the long-term efficacy of the Seresto® collar against a range of tick species world-wide in dogs and cats. Some of these studies have also presented data demonstrating a repellency effect, employing various protocols. Stanneck et al. (2012a) demonstrated the acaricidal efficacy of Seresto® (100% for 8 months duration) against the Brown dog tick *Rhipicephalus sanguineus* after 24 h incubation with hair from treated dogs and cats. Efficacy and repellency against several tick species were shown by Stanneck et al. (2012b). Sustained acaricidal (48 h) efficacy, for 8 months was 100% against *I. ricinus* (in vivo), and 100% against *I. scapularis* (in vitro), above 97% against *R. sanguineus*, generally above 97% against *Dermacentor reticulatus* and above 90% for *D. variabilis*.

Stanneck et al. (2012b) also measured the repellency effect by placing ticks directly on to the dogs and counting ticks at 6 h post-infestation, using a protocol design based on the European Medicines Agency (EMEA) guideline (2007). 6 h post-infestation counts were chosen for this trial to provide evidence for interference of transmission of VBDs, as transmission of *Ehrlichia canis* had been shown to occur within a few hours of tick attachment (Fourie et al. 2013). The EMEA guideline was subsequently updated in 2015 to include further advice on measurement of repellency for acaricides, based on Marchiondo et al. (2013). Stanneck et al. (2012b) demonstrated that repellency, based on tick counts done at 6 h post-infestation, was consistently 100% against *I. ricinus,* and above 90% against *R. sanguineus* for 8 months. Efficacy and repellency of the Seresto® collar against ticks in cats was also confirmed by Stanneck et al. (2012c). Sustained acaricidal (48 h) efficacy over a period of eight months was consistently 100% against *I. ricinus*, >98.5% against *Amblyomma americanum* and >94% against *R. turanicus*. Repellent (6 h), efficacy (protocol also based on the EMEA 2007 guideline) was consistently 100% for 8 months against *I. ricinus*, and between 54.8% and 85.4% against *R. turanicus*.

Dantas-Torres et al. (2013) further proved the repellent effect of the Seresto® collar against ticks endemic to Italy, by demonstrating the efficacy of the collar

in preventing transmission of VBD pathogens. The overall efficacy for the prevention of transmission of the canine tick-borne pathogens *Anaplasma platys* and *Babesia vogeli* by *R. sanguineus*, was 91.6 % (100 % for *B. vogeli,* 91.1 % for *A. platys*). In addition, Stanneck et al. (2013) confirmed the long term protection by Seresto® against transmission of *E. canis* to dogs by *R. sanguineus*. Over the 12 month study none of the collar-treated dogs were infected, whereas 34 of the 35 untreated dogs were infected. This study emphasised the importance of the repellency effect for tick control products. In Australia, vector borne pathogens including *B. vogeli*, *A. platys* and haemotropic *Mycoplasma* spp., have been shown to affect the health of dogs (Hii et al. 2012, 2015).

In light of the highly toxic nature of *I. holocyclus* in domestic animals, acaricides must demonstrate a very high level of efficacy (> 95 %) in order to achieve product registration in Australia. Smith et al. (2013) demonstrated 4 month efficacy (> 97.9 %) for the Seresto® collar against the *I. holocyclus*. However, repellency was not assessed in this study.

The lack of agreed, standardised methods for the evaluation of tick repellency in dogs and cats was highlighted by Halos et al. (2012) and harmonised methods were suggested. This paper reviewed the definition of the term 'repellent effect' in relation to acaricides for use in veterinary medicine. Two types of repellency effect were described. Repellency *sensu stricto* was defined as the effect of an acaricide that causes ticks to avoid the treated animal, or fall off when they come into contact with the hair coat. These authors recommended the measurement of the absence of ticks on treated animals compared to non-treated controls between 0 and 4 h post-challenge, and the use of 'infestation chambers' to collect and count ticks off-host. The World Association for Advancement of Veterinary Pathology (WAAVP) guidelines for evaluation of the treatment, prevention and control of ticks and fleas (Marchiondo et al. 2012) further refined this method. The guideline proposed placing sedated, treated animals in infestation chambers, releasing ticks beside the animals and counting the ticks remaining in the chambers at 30 min to 1 h post-challenge. This method is useful for measuring repellency for ixodid ticks (such as *I. holocyclus*) as ticks of this genera have been shown to move towards potential hosts over short distances, attracted by body heat, odours and increased CO_2 concentrations of the host (Marchiondo et al. 2013).

Halos et al. (2012) defined repellency *sensu lato* (expellency) as the combined effect of disruption of tick attachment and causing ticks to fall off the host within 24 h of exposure. In the present study, this effect was proven by a significant ($p < 0.002$) reduction in the number of ticks attached to dogs (ToA) in the treated group compared with the untreated controls at 6 and 24 h post-challenge. Marchiondo et al. (2013) recommended a protocol for measuring repellency *sensu lato* that involved placing ticks onto sedated animals in infestation crates and assessing ToA for periods up to 24 h. In the present study ticks were initially placed next to sedated animals, however repellency *sensu lato* can still be inferred from this study, as the number of ticks that did move towards and attach to the untreated control animals was high (geometric mean ToA for the untreated controls was over 19 of the 50 ticks [minimum 38 %] released beside the dogs throughout the study (Table 4)). Marchiondo et al. (2013) recommended a tick retention rate of at least 20 % on non-treated animals would be generally acceptable for inclusion of animals in efficacy studies for acaricides.

Finally, the killing effect, or overall efficacy of Seresto® was measured in this study by ToL counts at 6 and 24 h post-challenge, as recommended by Marchiondo et al. (2013). The excellent efficacy (killing effect) of the Seresto® collar against *I. holocyclus* for 6 months after treatment was confirmed in this study (consistently > 95 %), measured at 6 h post-challenge.

Limitations of the present study include that only one study site, with ticks collected from one general locality were incorporated in this study. Ticks collected from different regions within the ticks' geographic range would have provided more complete information on the repellency of the Seresto® collar for this species. Secondly, the application of this novel method for determination of repellency carried the risk that only limited numbers of ticks would attach to the untreated dogs. However, the attachment of ticks to control dogs was adequate, underpinning the robustness of the data. Finally, based on the efficacy and repellency results for other species of ticks globally up to 8 months, it would have been informative if the current study could also have been extended to 8 months.

Recommendations for further research arising from this study include replicating this protocol in cats. Positive results would be of great benefit as currently there are a very limited number of acaricides available for tick control in cats, and many Australian cats are paralysed every year by *I. holocyclus*.

Conclusions

This study demonstrated the excellent repellency effect of the Seresto® collar over a 6 month period against the Australian paralysis tick in dogs, using both definitions by Halos et al. (2012): *sensu stricto* and *sensu lato* (expellency). The repellency effect plays a significant role in the overall efficacy of the collar and offers great benefits in preventing the consequences of bites of *I. holocyclus* in dogs including paralysis, transmission of vector-borne diseases, blood loss, pain and irritation and secondary skin infections.

Ethical Standards

This study was performed in compliance with the NSW Department of Primary Industry Animal Welfare Branch and the Australian Pesticides and Veterinary Medicines Authority General Research Permit PER7250.

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Conflict of Interest

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