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Comparative Efficacy of an Imidacloprid/Flumethrin Collar (Seresto®) and an Oral Fluralaner Chewable Tablet (Bravecto®) against Tick (*Dermacentor variabilis* and *Amblyomma americanum*) Infestations on Dogs: a Randomised Controlled Trial

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Abstract

This controlled laboratory study demonstrated the residual speed of efficacy of an imidacloprid/flumethrin collar (Seresto®, Bayer) for the control of ticks (*Dermacentor variabilis, Amblyomma americanum*) at 6 and 12 hours post-infestation on dogs when compared to oral fluralaner (Bravecto®, Merck). Dogs were randomised by pre-treatment tick counts: Group 1) imidacloprid 10 % (w/w)/flumethrin 4.5 % (w/w) collar, 2) fluralaner (dosage 25.1–49.4 mg/kg), and 3) non-treated controls. Ticks (50/species/dog) were infested on days 3, 14, 21, 28, 42, and 56 followed by 50 *D. variabilis* on days 70 and 84. Live and dead attached ticks were counted 6 and 12 hours later. Efficacy against both species at 6 and 12 hours for Group 1 was 94–100 %. Efficacy for Group 2 against both species at 6 hours was 4–69 %; efficacy at 12 hours was 8–100 %. Live (attached and non-attached) tick counts at 6 hours in Group 1 were significantly lower ($p \leq 0.05$) than counts in Group 2 and 3 on all days. At 12 hours, live counts were significantly lower ($p \leq 0.05$) in Group 1 than Group 2 for *D. variabilis* from days 56–84 and for *A. americanum* from days 28–56. There were significantly fewer ($p \leq 0.05$) total ticks (total live and dead attached) on dogs in Group 1 compared to Group 2 and 3 at all time points. This study demonstrated that an imidacloprid/flumethrin collar was highly efficacious (94–100 %) at repelling and killing ticks on dogs at 6 and 12 hours post-infestation and was more efficacious than fluralaner as early as 6 hours post-infestation on all challenge days.

Fig. 1 Male (below) and female (above) *Dermacentor variabilis*

Fig. 2 Male (below) and female (above) *Amblyomma americanum*

Introduction

Ticks are persistent and hardy arachnids that pose a danger to humans and their pets through tick-borne diseases. The myriad of tick species and wide variety of tick lifestyles makes them difficult to control in domestic animals, and their ability to survive adversity allows them to successfully follow their hosts into new and diverse habitats. Those traits, along with other factors such as habitat destruction, climate change, and pet portability, will likely increase encounters between ticks and pets in the future (Bacon et al. 2008; Chomel 2011; Diuk-Wasser et al. 2012; Hamer et al. 2010). New information is continually being learned regarding tick-borne diseases in the medical and veterinary communities. For instance, ticks carry many different vector-borne diseases, and it is now known that a single tick may carry and transmit more than one pathogen at each feeding (Williams-Newkirk et al. 2014). New tick-borne pathogens have also been discovered, such as the Bourbon virus in 2014 and the Heartland virus in 2012 (Kosoy 2015; McMullan et al. 2012). Also, older tick-borne organisms, previously thought to be non-pathogenic, are now being considered as pathogenic (Krause et al. 2015). Furthermore, traditional transmission times of some tick-borne illnesses, such as Lyme disease, are being questioned and may be shorter than previously considered (Cook 2015). All of these factors make acaracide kill times especially important in the prevention of tick-borne diseases as there are significant risks involved with the feeding of even one tick.

The imidacloprid/flumethrin collar (Seresto®, Bayer) was introduced in the United States in 2013. It is an innovative flea and tick collar with unique technology to protect dogs and cats from fleas and ticks for 8 months. The active ingredients in the collar are released slowly onto the skin and hair of the pet, providing both repel and kill properties through contact with the active ingredients. Therefore, fleas and ticks do not have to bite or attach in order to die, an important feature in the prevention of vector-borne diseases. Also, the 8 month duration of protection with only one application can aid in owner compliance through ease of use. Fluralaner (Bravecto®, Merck) is another commercially available product that kills fleas and ticks; however, there is no evidence for repellent activity. Oral products, with a systemic distribution, require both fleas and ticks to penetrate the skin and initiate the feeding process in order to contact the active ingredient. This may enable

transmission of disease-causing organisms before the parasite is killed. This randomised controlled comparative laboratory study was designed to evaluate the residual speed of efficacy of Seresto® for the control of *Dermacentor variabilis* (Fig. 1) and *Amblyomma americanum* (Fig. 2) ticks at 6 and 12 hours post-infestation on dogs when compared to 2 competitive products and a non-treated negative control; results from 3 groups are reported here.

Materials and methods

Animals

Dogs were included in the study if they were over 6 months of age, determined to be healthy based on physical examination, not pregnant, were able to harbor an adequate tick infestation, and were not exposed to a previous insecticide/acaracide within 90 days of study onset. Forty-eight dogs were initially evaluated for the ability to harbor adequate tick infestations. All dogs were infested with approximately 50 *D. variabilis* on study day-7 with tick counts performed 48 hours later. Dogs were then ranked by live tick counts in descending order and randomised in sets of 4. The 8 dogs with the lowest tick counts were not included in the study. The results from 3 groups of 10 animals, Group 1) Seresto®, Group 2) Bravecto®, and Group 3) non-treated controls, are reported here. The fourth group, afoxolaner (NexGard®, Merial), along with dogs in Groups 1 and 3 are presented in a separate manuscript.

Products

Both products were administered according to the label directions and weight of each dog prior to treatment. On day 0, Seresto® collars, imidacloprid 10 % (w/w)/flumethrin 4.5 % (w/w), were applied to all dogs in Group 1. The length was adjusted, in accordance with the label, and the extra length cut off and secured with a ratchet mechanism. Collars were applied according to the dog's bodyweight (collar dose ranges: small collar < 18 lbs, large collar > 18 lbs). Group 2 dogs were treated orally with Bravecto® at a dosage of 25.1 – 49.4 mg/kg bodyweight. Two size chewable tablets were available and dosed based on bodyweight: 250 mg and 500 mg. The control group remained non-treated.

Experimental infestations

Dogs were infested with *D. variabilis* and *A. americanum* based on a predetermined schedule. Each infestation consisted of approximately 50 ticks of each species applied along the dog's dorsal midline from shoulders to hips.

Experimental design

This laboratory study was conducted in accordance with VICH GL9 Good Clinical Practices (GCP), June 2000 (FDA Guidance for Industry 85, May 2001) and applicable standard operating procedures. Dogs were housed individually in runs throughout the study. They were bathed with a mild, non-medicated shampoo, combed thoroughly, and allowed to acclimate for 11 days prior to product administration. Concomitant treatments were prohibited except where deemed necessary and would not influence the performance of any product. Following product administration, 50 *D. variabilis* and 50 *A. americanum* were infested per dog on days 3, 14, 21, 28, 42, and 56 followed by infestation with only 50 *D. variabilis* on days 70 and 84. This schedule was created due to the fact that fluralaner is only labeled against *A. americanum* for 8 weeks. Tick counts were performed at 6 hours (thumb count) and 12 hours (full body comb count with tick removal) following infestation to assess the speed of repellency and/or kill; all ticks on the dogs were counted including both live (attached and non-attached) and dead attached ticks.

Clinical monitoring

During acclimatisation, dogs were observed at least once daily. A physical examination was performed to verify health status during the acclimatisation period. For the remainder of the study, dogs were observed daily until study completion, at which

time all dogs were returned to the supplier. On study day 0, dogs were also observed 2 and 4 hours after product administration for adverse events.

Efficacy determination

Total live (attached and non-attached) and dead attached tick counts were determined and recorded. Individual live tick counts were used to calculate a geometric mean (GM) for each group at each time point on the specified study days. For each post-treatment tick count, efficacy was calculated using Abbott's Formula. Percent efficacy (% reduction) was determined by comparing the GM number of live ticks retained on the treated group to the GM number of live ticks retained on the non-treated negative control group using the following formula:

$$\% \text{ efficacy} = \frac{\text{GM tick count control} - \text{GM tick count (treatment)}}{\text{GM tick count(control)}}$$

Data analysis

For *D. variabilis*, the assumption of equally distributed tick-ridding ability was assessed by descriptively summarising the pre-study tick counts. The statistical method comparing post-treatment tick counts utilised the pre-study tick counts for each animal. The efficacy of the treated groups, relative to the control group, was computed with Abbott's formula. Arithmetic mean counts and geometric

Table 1 *Dermacentor variabilis* efficacy and geometric mean live (attached and non-attached), dead attached, and total (total live and dead attached) tick counts after treatment with Seresto® or Bravecto®

Study Day	Day 3		Day 14		Day 21		Day 28		Day 42		Day 56		Day 70		Day 84	
Hours post-infestation	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h
Seresto®																
Mean # live	1.5^{c}	1.0^{b}	0.0^{c}	0.0^{b}	0.2^{b}	0.0^{b}	0.1^{b}	0.1^{b}	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{b}	0.1^{b}	0.0^{b}
Mean # dead attached	0.1^{ab}	1.1^{a}	0.8^{ab}	0.5^{a}	0.3^{a}	0.5^{a}	0.2^{a}	0.2^{a}	0.1^{a}	0.1^{a}	0.2^{a}	0.2^{a}	0.9^{b}	0.3^{ab}	0.3^{a}	0.0^{a}
Mean # total	1.6^{b}	2.1^{b}	0.8^{b}	0.5^{c}	0.4^{b}	0.5^{b}	0.2^{b}	0.2^{c}	0.1^{b}	0.1^{c}	0.2^{b}	0.2^{b}	0.9^{b}	0.3^{b}	0.4^{b}	0.0^{b}
% Efficacy^{d}	94.9	97.0	100	100	99.0	100	99.7	99.8	100	100	100	100	100	100	99.6	100
Bravecto®																
Mean # live	9.1^{b}	0.6^{b}	5.9^{b}	0.0^{b}	7.4^{a}	0.3^{b}	13.5^{a}	0.9^{b}	14.1^{a}	0.7^{b}	12.3^{a}	3.9^{b}	16.4^{a}	10.5^{a}	19.8^{a}	25.8^{a}
Mean # dead attached	1.9^{b}	11.3^{b}	2.5^{b}	10.0^{b}	1.1^{a}	9.2^{b}	0.2^{a}	9.9^{b}	0.1^{a}	8.3^{b}	0.2^{a}	5.7^{b}	0.0^{a}	2.3^{b}	0.2^{a}	0.3^{a}
Mean # total	12.6^{a}	12.4^{a}	9.2^{a}	10.0^{b}	10.8^{a}	9.9^{a}	13.8^{a}	11.5^{b}	14.3^{a}	9.5^{b}	12.5^{a}	11.2^{a}	16.4^{a}	16.0^{a}	20.1^{a}	27.1^{a}
% Efficacy^{d}	68.0	98.3	68.6	100	62.7	98.8	35.4	96.8	20.7	97.2	14.7	82.5	12.4	62.7	5.0	8.5
Control																
Mean # live	28.5^{a}	34.6^{a}	18.9^{a}	29.2^{a}	19.8^{a}	29.0^{a}	20.9^{a}	29.6^{a}	17.8^{a}	26.2^{a}	14.5^{a}	22.4^{a}	18.8^{a}	28.1^{a}	20.9^{a}	28.1^{a}
Mean # dead attached	0.0^{a}	0.0^{a}	0.2^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Mean # total	28.5^{a}	34.6^{a}	19.3^{a}	29.2^{a}	19.8^{a}	29.0^{a}	20.9^{a}	29.6^{a}	17.8^{a}	26.2^{a}	14.5^{a}	22.4^{a}	18.8^{a}	28.1^{a}	20.9^{a}	28.1^{a}

^{a,b,c} Values down columns with unlike superscripts are significantly different ($p \leq 0.05$)
^{d} % Efficacy calculated using Abbott's Formula
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

means were both used in the efficacy calculation. Geometric means were calculated following transformation using a logarithmic method (averaging the transformed values, and converting the average using antilog to represent a geometric mean). Because some animals might have had zero (0) tick counts, all counts were modified by adding one (1) to each prior to logarithmic transformation. Also, one (1) was subtracted from the antilog value to meaningfully represent the geometric mean for each group. Only live tick counts were used to calculate efficacy.

Live, dead attached, and total (total live and dead attached) tick counts for each species were analysed separately. Log (tick counts+1) were analysed with a repeated measures analysis of covariance (RMANCOVA) including terms for treatment (TRT), animal (random), study day (DAY), and the interaction of treatment and study day (TRT x DAY), using the pre-treatment tick counts as a covariate (for *D. variabilis* only, no baseline covariate when analysing for the other tick species). SAS PROC MIXED (SAS® Institute, Cary, NC) was used for analysis with the covariance structures 'AR(1)' and 'ARH(1)' for data collected on equal intervals, or 'CS' and 'CSH' for data collected on unequal intervals. Results from the model with the smallest Akaike's Information Criterion were used.

If the interaction of treatment and study day was significant at the 0.05 level, multiple group pairwise comparisons were generated using a Bonferroni alpha adjustment for multiple group comparisons.

Table 2 *Amblyomma americanum* efficacy and geometric mean live (attached and non-attached), dead attached, and total (total live and dead attached) tick counts after treatment with Seresto® or Bravecto®

Study Day	Day 3		Day 14		Day 21		Day 28		Day 42		Day 56	
Hours post-infestation	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h	6h	12h
Seresto®												
Mean # live	0.7[b]	0.2[b]	0.0[b]	0.0[b]	0.1[b]	0.1[b]	0.1[b]	0.0[c]	0.2[b]	0.0[b]	0.2[b]	0.0[b]
Mean # dead attached	0.2[a]	0.7[a]	1.1[a]	0.7[a]	0.7[a]	0.4[a]	0.3[a]	0.3[a]	0.4[a]	0.2[a]	0.4[a]	0.2[a]
Mean # total	1.0[b]	0.9[b]	1.1[b]	0.7[b]	0.7[b]	0.4[b]	0.4[b]	0.3[b]	0.7[b]	0.2[b]	0.5[b]	0.2[b]
% Efficacy[d]	94.2	98.4	100	100	99.6	99.7	99.5	100	98.7	100	98.8	100
Bravecto®												
Mean # live	5.4[a]	0.5[b]	5.6[a]	1.5[b]	8.3[a]	1.4[b]	9.8[a]	3.8[b]	11.5[a]	10.9[a]	13.1[a]	11.2[a]
Mean # dead attached	1.1[a]	9.4[b]	1.0[a]	8.4[b]	0.8[a]	10.7[b]	0.2[a]	10.6[b]	0.3[a]	4.8[b]	0.4[a]	2.9[b]
Mean # total	7.8[a]	10.5[a]	7.1[a]	10.7[a]	10.7[a]	13.1[a]	10.3[a]	16.1[a]	12.0[a]	18.8[a]	13.6[a]	14.8[a]
% Efficacy[d]	55.5	96.8	35.1	84.9	57.3	94.2	35.3	82.5	33.3	49.8	4.2	33.6
Control												
Mean # live	12.2[a]	14.4[a]	8.6[a]	9.6[a]	19.4[a]	23.4[a]	15.1[a]	21.8[a]	17.3[a]	21.8[a]	13.7[a]	16.8[a]
Mean # dead attached	0.0[a]	0.0[a]	0.12[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]	0.0[a]
Mean # total	12.2[a]	14.4[a]	8.8[a]	9.6[a]	19.4[a]	23.4[a]	15.1[a]	21.8[a]	17.3[a]	21.8[a]	13.7[a]	16.8[a]

[a,b,c] Values down columns with unlike superscripts are significantly different ($p \leq 0.05$)
[d] % Efficacy calculated using Abbott's Formula
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

Table 3 Sequence of events leading to successful feeding of ticks (Anderson and Magnarelli 2008)

Appetence	Hunting or seeking host
Engagement	Adherence to the skin or fur of the host
Exploration	Searching on the skin for suitable attachment site
Penetration	Insertion of the mouthparts into the host's epidermis and dermis
Attachment	Feeding site established
Ingestion	Uptake of blood and other fluids
Engorgement	Partial or complete meals of blood taken
Detachment	Withdrawal of the mouthparts
Disengagement	Tick drops off of the host

These simple effect pairwise comparisons were obtained from the TRT x DAY interaction. If the interaction term was not significant ($p > 0.05$), the TRT main effect was evaluated. If the TRT main effect was not significant ($p > 0.05$), the results were deemed not significant and no further analyses were conducted. If the TRT main effect was significant ($p \leq 0.05$), multiple group pairwise comparisons were generated using a Bonferroni alpha adjustment for multiple group comparisons across the pooled time points. In addition, dead attached ticks and total ticks were compared across groups. However, pre-treatment *D. variabilis* counts were not used as a covariant when dead attached or total ticks were analysed. All four treatment groups were analysed together, however only the results from Groups 1, 2 and 3, are reported here. Software from SAS® Institute, Cary, NC version 9.3 was used for all analyses.

Results

Thirty dogs (11 female and 19 males) were included in Groups 1, 2 and 3. All dogs were over 6 months of age and ranged in weight from 5.3–15.2 kg. Dogs were dosed according to the label directions for each product. In Group 1, there was 1 dog treated with the small dog Seresto® collar (< 18 lbs) and 9 dogs were treated with the large dog collar (> 18 lbs). In Group 2, the Bravecto® dosage ranged from 25–49.3 mg/kg. There were no adverse events during the study. One dog in Group 1 was removed on study day 34 due to the fact that the dog removed and destroyed its collar.

Efficacy against *D. variabilis* at 6 hours for Group 1 was 95–100 % and for Group 2 was 5–69 % (Table 1, Fig. 3); efficacy at 12 hours for Group 1 was 97–100 % and for Group 2 was 8–100 % (Table 1, Fig. 4). Efficacy against *A. americanum* at 6 hours for Group 1 was 94–100 % and for Group 2 was 4–57 % (Table 2, Fig. 5); efficacy at 12 hours for Group 1 was 98–100 % and for Group 2 was 34–97 % (Table 2; Fig. 6). The majority of live ticks on the Bravecto treated dogs at 6 hours post-infestation were attached to the dogs and not loose on the body (average for *D. variabilis*: 58 %–99 % and *A. americanum*: 77–95 %); at 12 hours, an average of > 99 % of live ticks on Bravecto dogs were attached and feeding (except day 3 which was 40 %). Live (attached and non-attached) tick counts at 6 hours for both species in Group 1 were significantly lower ($p \leq 0.05$) than counts in Group 2 and 3 on all challenge days. At 12 hours, live *D. variabilis* counts were significantly lower ($p \leq 0.05$) in Group 1 as compared to Group 2 from days 56–84 and against *A. americanum* from days 28–56. Also, there were significantly fewer ($p \leq 0.05$) total ticks (total live and dead attached) of each species on the dogs in Group 1 compared to Group 2 and 3 at all time points (Table 1–2, Fig. 7–10). When the two species were combined (sum total of average of *D. variabilis* and *A. americanum*), total tick counts for Group 1 were at most only 3.0 ticks/dog, whereas Group 2 reached 28.3 ticks/dog, and Group 3 reached 52.4 ticks/dog.

Discussion

In this study, Seresto® was consistently efficacious over the entire study period at both time points and for both tick species, exhibiting a rapid speed of contact kill as well as repellency. Additionally, no adverse events were reported for Seresto®, further

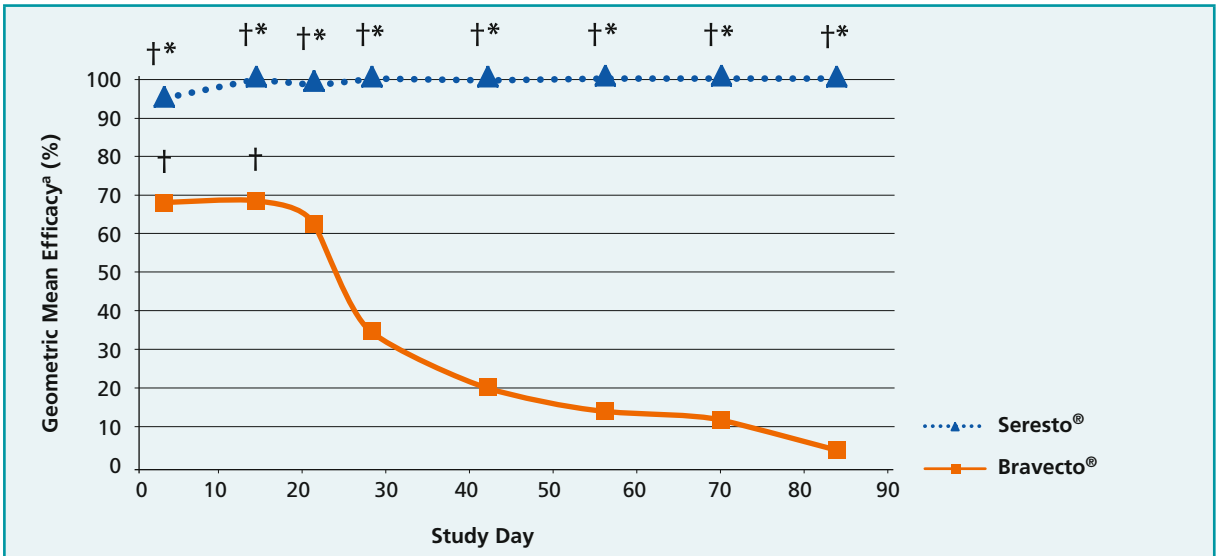

Fig. 3 Efficacy 6 hours post-infestation with *D. variabilis* after treatment with Seresto® or Bravecto®
* Live tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Live tick counts significantly different ($p \leq 0.05$) as compared to control group
[a] % Efficacy calculated using Abbott's Formula

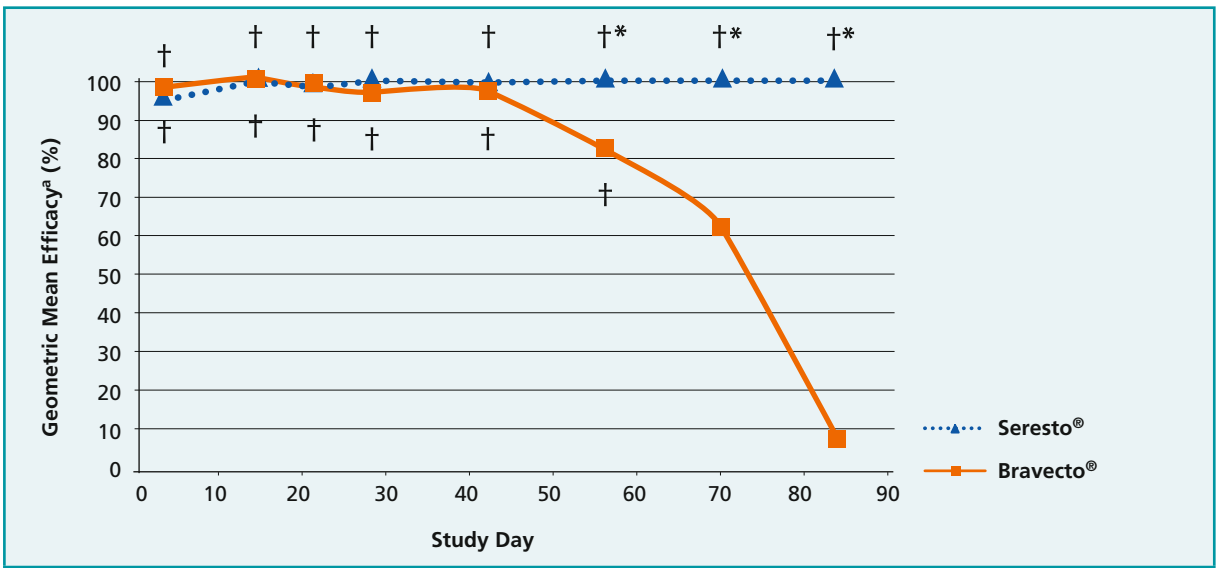

Fig. 4 Efficacy 12 hours post-infestation with *D. variabilis* after treatment with Seresto® or Bravecto®
* Live tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Live tick counts significantly different ($p \leq 0.05$) as compared to control group
[a] % Efficacy calculated using Abbott's Formula

supporting the safety of the product. Results from previous Seresto® efficacy studies agree with the results found here. In a field trial, in which dogs were naturally infected with ticks, Seresto® treated dogs had a high percentage of efficacy ranging from 91.2–100 % over the 8 month period (Stanneck et al. 2012). In a laboratory study, in which dogs were repeatedly re-infested with *Rhipicephalus sanguineus*, the protective 6 hour efficacy of Seresto® was 85.6 % on day 7 and 90.1–97.1 % from day 14 to

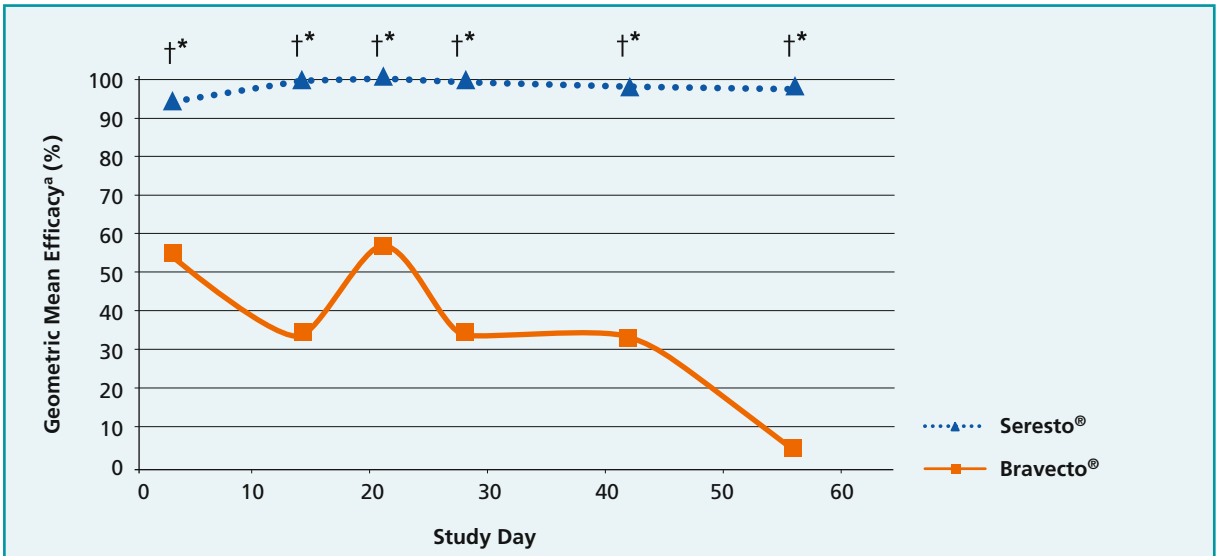

Fig. 5 Efficacy 6 hours post-infestation with *A. americanum* after treatment with Seresto® or Bravecto®
* Live tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Live tick counts significantly different ($p \leq 0.05$) as compared to control group
[a] % Efficacy calculated using Abbott's Formula

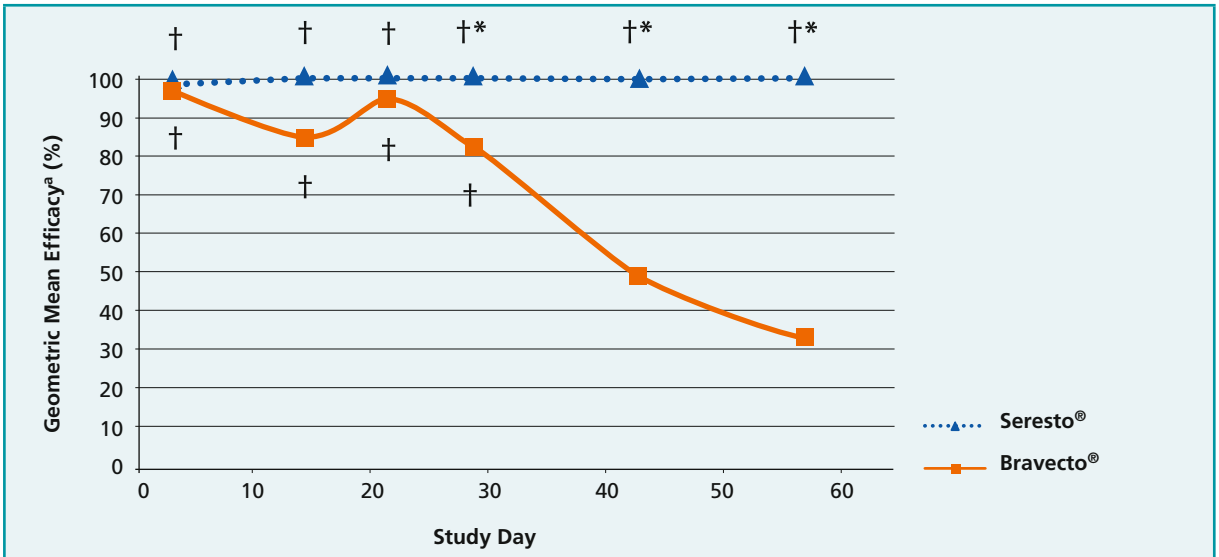

Fig. 6 Efficacy 12 hours post-infestation with *A. americanum* after treatment with Seresto® or Bravecto®
* Live tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Live tick counts significantly different ($p \leq 0.05$) as compared to control group
[a] % Efficacy calculated using Abbott's Formula

the end of the study (day 70). In the same study, the protective 18 hour tick efficacy was 98–99.6% during the entire study period (Horak et al. 2012). The results presented here illustrate the rapid repellent properties of Seresto® by inhibiting almost all ticks from attaching and feeding within 6 hours of infestation. Ticks that are repelled and killed are unable to attach and feed, therefore decreasing the risk of disease-causing organism transmission. The authors' have experienced the

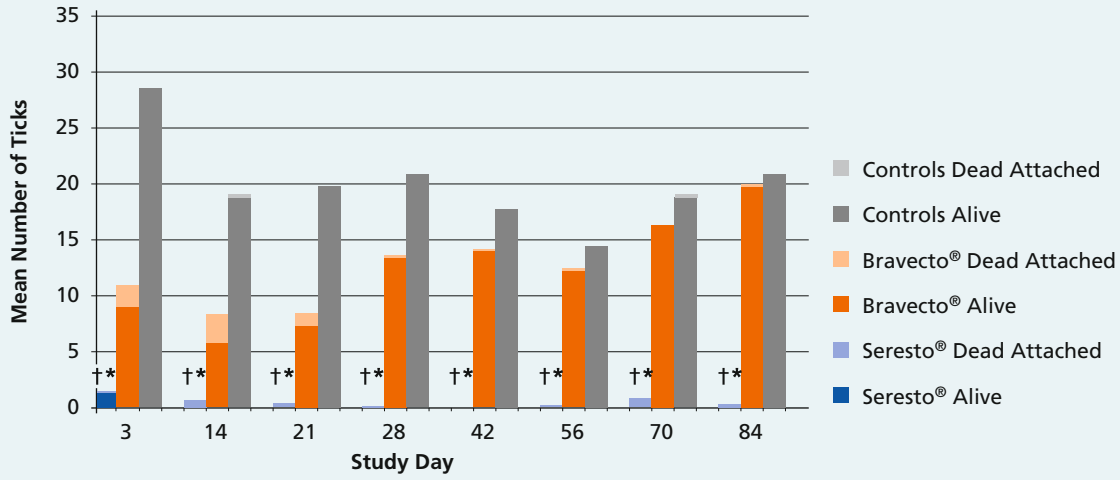

Fig. 7 *Dermacentor variabilis* 6 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or Bravecto®
* Total tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Total tick counts significantly different ($p \leq 0.05$) a compared to control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

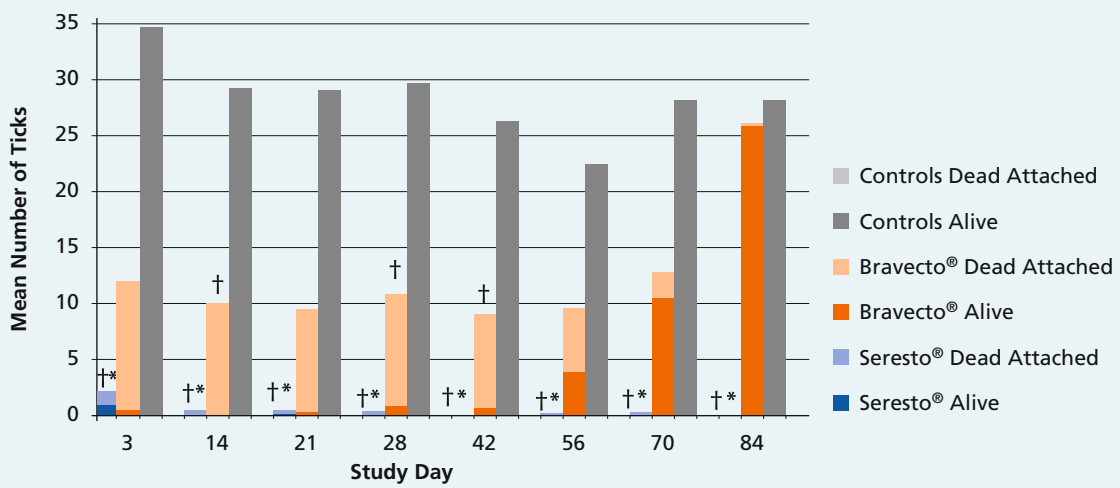

Fig. 8 *Dermacentor variabilis* 12 hour geometric mean live (attached and non-attached) and dead tick counts after treatment with Seresto® or Bravecto®
* Total tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Total tick counts significantly different ($p \leq 0.05$) as compared to control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

same repellent properties in clinical observations. Ticks on Seresto® treated dogs appear uncomfortable and irritated almost immediately and are found crawling aimlessly on the haircoat until they fall off of the dog and are killed. The World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines further characterised this repellent action (Marchiondo et al. 2013). These guidelines characterize sensu stricto repellency with ticks that are found either moving away from

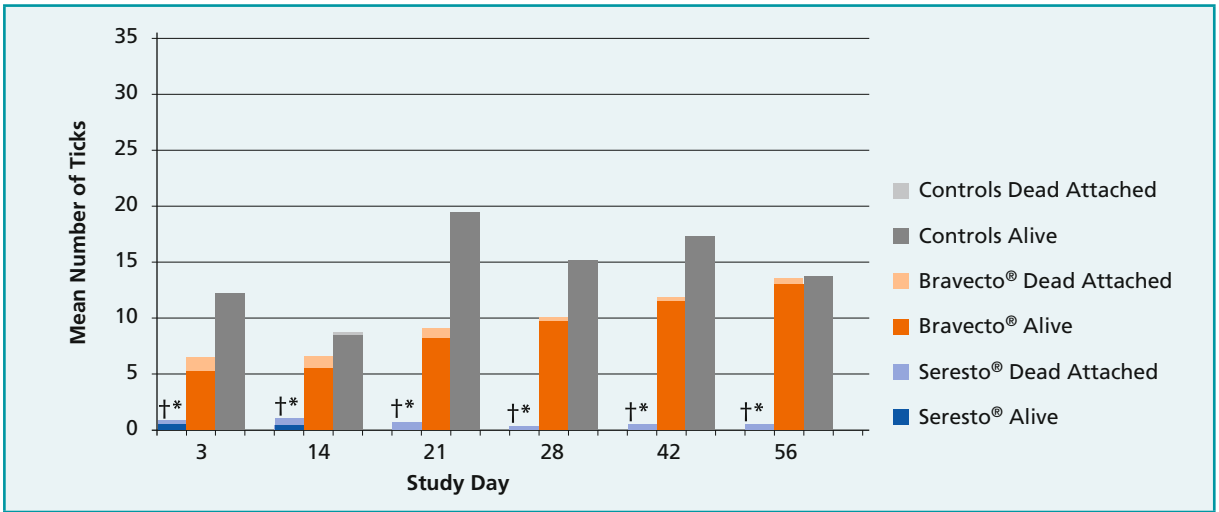

Fig. 9 *Amblyomma americanum* 6 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or Bravecto®
* Total tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Total tick counts significantly different ($p \leq 0.05$) as compared to control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

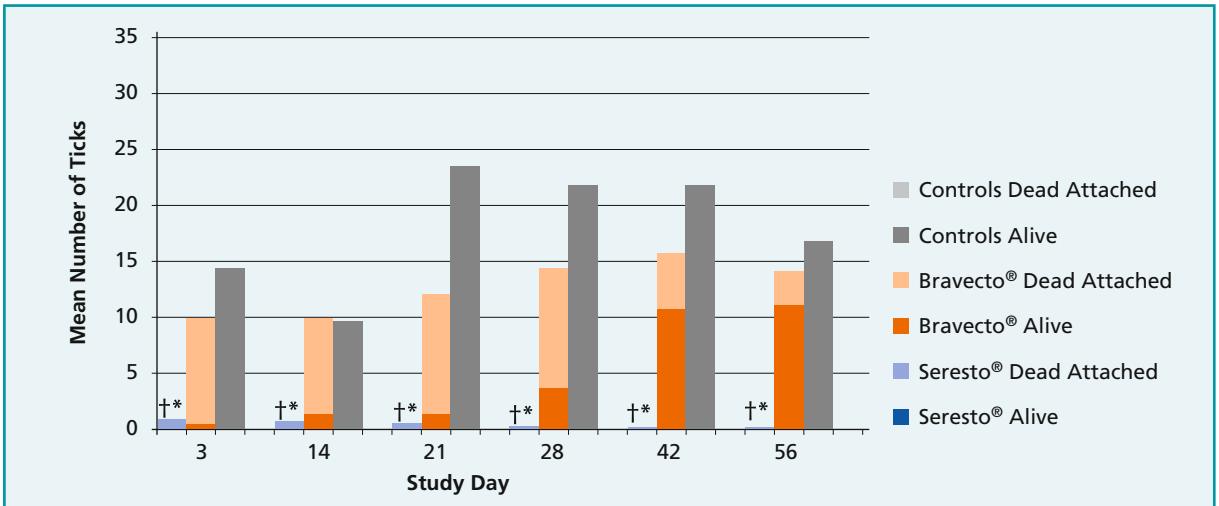

Fig. 10 *Amblyomma americanum* 12 hour geometric mean live (attached and non-attached) and dead attached tick counts after treatment with Seresto® or Bravecto®
* Total tick counts significantly different ($p \leq 0.05$) as compared to Bravecto® group
† Total tick counts significantly different ($p \leq 0.05$) as compared to control group
Note: Geometric Mean # totals are not expected to equal the sum of geometric mean # live + geometric mean # dead attached due to the mathematical basis for calculating geometric means.

the treated animal or that fall off within 6–8 hours after contact with treated haircoat, both characteristics of ticks applied to Seresto® treated dogs. Repellency and/or a rapid speed of kill are more important now than ever before. Ticks may transmit pathogens to hosts much faster than previously considered (Fourie et al. 2013b). Recommendations from the Canine Vector-Borne Disease world forum in 2011 (CVBD) note that pathogen transmission can occur almost immediately from

some ticks, and the concept of repellency is important to avoid blood meals by arthropod vectors (Baneth et al. 2012). Using a product with repellent properties, such as Seresto®, can inhibit ticks from attaching and feeding, therefore aiding in the prevention of disease transmission. While this was not a disease transmission study, several studies have successfully confirmed the ability of Seresto® to help prevent the transmission of disease-causing organisms to dogs and cats including: *Ehrlichia canis, Babesia canis, Babesia vogeli, Anaplasma platys,* and *Cytauxzoon felis* (Dantas-Torres et al. 2013; Fourie et al. 2013a; Reichard et al. 2013; Stanneck and Fourie 2013).

A product that offers repellency and/or a rapid speed of kill may also reduce the likelihood of transmission of pathogens between ticks. Some species of tick-borne pathogens benefit from co-feeding, which allows the pathogen to be transmitted from tick to tick without systemic transmission to the host. First described in tick borne encephalitis virus transmission (Labuda et al. 1996), ticks who feed either in physical proximity or temporal proximity can share pathogens on the skin surface at bite sites, eliminating the need to transmit the pathogen to the host first. The importance of this transmission process is still being explored in other pathogens (Harrison and Bennett 2012). Tick prevention products with rapid contact repellency and/or kill have the best chance of preventing the transmission of pathogens between ticks in either systemic or co-feeding paradigms. This will in turn, reduce the number of infected ticks in the environment and therefore, the risk of tick-borne infections in humans and pets.

There are 9 steps that occur during the feeding process of a tick (Table 3) (Anderson and Magnarelli 2008). For orally administered, systemically active products, such as Bravecto®, the tick must penetrate the skin with its mouthparts and ingest the active ingredient through the hypostome (Fig. 11) from the vascular compartment of the dog in order to die; therefore, the feeding process is not interrupted until steps 5–7 (attachment, ingestion, and engorgement). On the other hand, products that repel ticks can target the feeding process as early as steps 2–3 (engagement and exploration). This difference in disruption of the feeding process between topically versus systemically active products is reflected in this study by the total (total live and dead attached) tick counts on the dogs. Dogs treated with Seresto® had significantly fewer total ticks on them at 6 and 12 hours post-infestation on all challenge days compared to dogs treated with Bravecto®. When the 2 species were combined, the sum of the mean total number of ticks on the Seresto® treated dogs was at most only 3.0 ticks/dog compared to 28.3 ticks/dog in the Bravecto® group. The repellent activity of Seresto® affected the feeding process earlier, significantly reducing the number of ticks on the dogs and the number of ticks that could penetrate the skin and attach compared to dogs treated with Bravecto®.

Fig. 11 *Amblyomma americanum* mouthparts (hypostome, chelicerae, and palps

No repellent activity was observed in dogs treated with Bravecto®, which would be expected with a systemically active product. At 6 hours post-infestation, most of the ticks on the dogs remained alive. During the first part of the study, a large portion of those ticks were dead at 12 hours, however, they remained attached to the dogs. Over time, the ticks weren't killed within 12 hours. The number of live attached ticks on Bravecto® treated dogs 12 hours

post-infestation increased with a corresponding decrease in efficacy down to 8.5 % for *D. variabilis* (at 12 weeks) and 33.6 % for *A. americanum* (at 8 weeks). This decrease in efficacy of Bravecto® over a 12 week study period was also observed 24 hours post-infestation with *Dermacentor reticulatus* (a species not found in North America) and *R. sanguineus* (Beugnet 2015). It can be presumed that as the active ingredient is cleared from the body, the plasma levels decrease and the feeding time required to kill a tick increases. The longer a tick remains attached and feeds on a dog, the higher the risk for transmission of disease-causing organisms. Attached and feeding ticks are not only a serious concern due to the pathogens they can transmit, they can also lead to other medical and cosmetic issues. Tick bites can lead to irritation and pruritus at the attachment site, secondary infections requiring medical treatment, and a variety of toxic or allergic reactions, some of which can be life threatening (CAPC 2011; CDC 2014). Treatment for these signs will be an additional expense for owners and additional stress for the pet. Attached ticks on a pet can also harm the human animal bond, but more importantly, can be a potential concern for owners in terms of exposure to disease-causing organisms during the removal and discard process if proper precautionary measures are not taken.

It should be noted that the WAAVP guidelines suggest that tick efficacy be determined 48 hours after an infestation and in special circumstances (systemic products or when taking into account the potential transmission of pathogens) efficacy determination may be delayed until 72 hours (Marchiondo et al. 2013). Therefore, despite the risk of transmission of disease-causing organisms within these long time periods, the purpose of this study was not to discredit the actual label claims for Bravecto®. The purpose was to determine the speed of repellency and/or kill of both products. Further studies should be performed to determine the effect of Bravecto® treatment on disease transmission.

Conclusion

This study demonstrated that Seresto® (an imidacloprid/flumethrin collar) was highly efficacious (94 – 100 %) at repelling and killing *D. variabilis* and *A. americanum* ticks on dogs at 6 and 12 hours post-infestation and was significantly more efficacious ($p \leq 0.05$ based on live tick counts) than Bravecto® (fluralaner) at the earlier time point (6 hours). The efficacy of Seresto® was also significantly higher ($p \leq 0.05$ based on live tick counts) than Bravecto® 12 hours post-infestation against *D. variabilis* after week 6 and against *A. americanum* after week 3. This was due to the longer amount of time required to kill ticks, resulting in an increase in the number of live attached ticks on the dogs. Also, there were significantly fewer total (total live and dead attached) ticks ($p \leq 0.05$) on the Seresto® treated dogs compared to the Bravecto® treated dogs at all time points, further illustrating the rapid repel and kill properties of Seresto®.

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Ethical standards:

The study was performed in compliance with current national laws and regulations.

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Conflict of interest

Cameon M. Ohmes, Joe Hostetler, Wendell Davis, Amy McMinn, and Terry Settje are employed by Bayer Health Care Animal Health. Terry Settje performed the statistical analyses. William Russell Everett is a consultant for Bayer Health Care Animal Health.

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