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Long-Term Efficacy of an Imidacloprid 10%/Flumethrin 4.5% Polymer Matrix Collar (Seresto®, Bayer) against the Australian Paralysis Tick (*Ixodes holocyclus*) in Dogs

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Abstract

Two placebo-controlled pen studies were conducted to assess the efficacy of an imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto®, Bayer; Investigational Veterinary Product (IVP)) against the Australian paralysis tick (*Ixodes holocyclus*). Dogs assigned to the placebo (n=8) or IVP (n≥8) groups had collars (placebo or IVP) attached on Day 0 and were infested with 30 unfed, adult, female *I. holocyclus* at 14–28 day intervals over 227 days. Ticks were counted 24, 48 and 72 h post infestation to determine the acaricidal efficacy of the IVP. The acaricidal efficacy of the IVP 72 h post infestation exceeded 95% on Days 17 (99.3%), 59 (99.7%), 73 (96.6%), 87 (100.0%), 101 (96.4%), 115 (99.1%) and 171 (95.8%), but dropped on Days 45 (94.0%) and 143 (77.8%), and declined from Day 199 (79.9%) to 227 (65.5%). No adverse events related to treatment were observed. This study has demonstrated the excellent acaricidal efficacy (97.9%) of the IVP collar against *I. holocyclus* 72 h post infestation over 16 weeks.

Introduction

The adult, female Australian paralysis tick (*Ixodes holocyclus*) injects a neurotoxin (holocyclotoxin) into its host as it feeds, with a single tick capable of causing paralysis and death in domestic animals including dogs and cats. This increases the importance of effective tick control along the eastern coast of Australia, where *I. holocyclus* is endemic.

The incidence of tick paralysis in domestic animals is highest in spring and summer, when adult ticks are most abundant, but can occur year-round in parts of the country and when environmental conditions favour their development. Currently available options for the control of *I. holocyclus* on dogs are limited and include topically applied spot-ons or sprays containing permethrin or fipronil that require fortnightly reapplication, or collars containing flumethrin, amitraz or deltamethrin that give protection for 6 weeks to 3 months.

Seresto® (Bayer; Investigational Veterinary Product (IVP)) contains imidacloprid (10%), an insecticide from the chloronicotinyl group of compounds, and flumethrin (4.5%), an ectoparasiticide belonging to the α-cyano-pyrethroids, in a novel polymer matrix collar that allows for their slow and continuous release onto the skin surface and hair coat of a treated animal (Stanneck et al. 2012a). The IVP is registered for use on dogs and cats in many countries including in the European Union, the United Kingdom, the United States of America and New Zealand. It has been shown to be efficacious for 8 months against fleas (*Ctenocephalides felis felis*) and ticks (*Amblyomma americanum*, *Dermacentor reticulatus*, *Dermacentor variabilis*, *Ixodes ricinus*, *Ixodes scapularis*, *Rhipicephalus sanguineus* and *Rhipicephalus turanicus*) on dogs and cats and additionally eliminates lice (*Trichodectes canis*) and mites (*Sarcoptes scabiei*) on dogs (Stanneck et al. 2012b; Stanneck et al. 2012c). Both of the active ingredients in the IVP are found in ectoparasiticides currently registered for use on dogs in Australia. Imidacloprid is an efficacious insecticide (Epe et al. 2003; Hellmann et al. 2003) and an active ingredient in the topical spot-ons Advantage® (Bayer Australia Ltd), Advocate® (Bayer Australia Ltd) and Advantix® (Bayer Australia Ltd) and flumethrin is an efficacious acaricide found in the Kiltix® (Bayer Australia Ltd) collar.

The current study was conducted to determine the magnitude and duration of efficacy of the IVP against *I. holocyclus* in dogs.

Materials and methods

Study design

Two placebo-controlled pen studies were conducted at a contract research facility[1] to determine the efficacy of the IVP against *I. holocyclus* in dogs, from application of the collar on Day 0 until Day 94 (study 1) and Day 98 until Day 227 (study 2). See Table 1 for an overview of the experimental design.

Study animals

The study dogs (study 1, n=16; study 2, n=20) were mixed-sex foxhounds, 0.7–9.3 years of age, weighing 28–55 kg, with an average hair length of 15–20 mm over the lateral thorax and abdomen and were identified by numbered metal tags and implanted microchips. The dogs did not receive an acaricide in the 60–90 days prior to study commencement, but were vaccinated and treated with a broad-spectrum anthelmintic prior to the studies. No routine prophylactic treatments were administered during the studies. Dogs were fed a maintenance quantity of complete, dry dog food once or twice daily, raw bones occasionally and had access to clean water. The dogs in the first study had an acquired immunity to holocyclotoxin induced by frequent infestation with *I. holocyclus*. In the second study, the dogs assigned to the placebo group were hyperimmunised against holocyclotoxin by exposing them to gradually increasing numbers of unfed, adult, female *I. holocyclus* over a 6-week period prior to the first tick challenge on Day 98. Dogs in the IVP group had previously demonstrated hyperimmunisation to holocyclotoxin, but did not undergo re-immunisation prior to application of the collars on Day 0, due to the expected waning of immunity during the period from Day 0 until the commencement of tick infestations on Day 98.

At all times, IVP- and placebo-treated dogs were kept separate and study personnel followed

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Table 1 Overview of experimental design and activities

Day	Activity STUDY 1
–14	Selection of study dogs, dogs washed (Natural shampoo; Dermcare Vet Pty Ltd), placebo collars fitted to all dogs to condition them to wearing collars
–7	Tick carrying capacity (TCC) test commenced: attached 30 ticks to each dog
–5	Ticks counted, but left in place to boost immunity. Dogs ranked according to TCC. Allocation of dogs to treatment groups, groups to pens and dogs within groups to pens
–1	Ticks removed
0	Acclimation collars removed. Collars fitted to IVP (n=8) and placebo (n=8) dogs
14	Tick challenge: attached 30 ticks to placebo (n=8) and IVP (n=8) treated dogs
15	Counted ticks
16	Counted ticks
17	Counted and removed ticks
42	Tick challenge: attached 30 ticks to placebo (n=8) and IVP (n=8) treated dogs
43	Counted ticks
44	Counted ticks
45	Counted ticks, but ticks left in place on all dogs until 86 h
46	Counted and removed ticks at 86 h
70	Tick challenge: attached 30 ticks to placebo (n=8) and IVP (n=8) treated dogs
71	Counted ticks
72	Counted ticks
73	Counted and removed ticks
84	Tick challenge: attached 30 ticks to placebo (n=8) and IVP (n=6) treated dogs
85	Counted ticks
86	Counted ticks
87	Counted and removed ticks
94	Study 1 concluded

appropriate measures to prevent transfer of chemical between treated and control dogs. Dogs were exercised in outdoor, grassed runs with other dogs from the same treatment group for at least 30 min on days that they were not infested with ticks and weather permitting. Dogs were acclimated to their pens for 7 days before the studies commenced. In the first study, the dogs were housed individually in pens from Day –7 to 94, except between Days 63 to 67 and 77 to 80, when the dogs in the placebo group were housed in pairs. This study commenced in summer (December) and finished in autumn (April), and the temperature in the pens ranged from 17 °C to 37 °C. In the second study, dogs from the same treatment group were housed in group pens of six to eight dogs between Days –7 to 91, and then in pairs between Days 91 to 227. This study commenced in early spring (September) and finished in autumn (May), and the ambient temperature ranged from 7 °C to 37 °C[2].

In the first study, dogs were ranked from greatest to least according to their tick-carrying capacity, which was tested on Day –7, and then assigned to the IVP or placebo group alternately. The dog with the greatest tick-carrying capacity was allocated to

[2] Temperature at closest station to Wongaburra Research Centre (Casino Airport; station 058208), recorded by the Australian Government, Bureau of Meteorology

Day	Activity STUDY 2
–7	Dogs washed (Natural shampoo; Dermcare Vet Pty Ltd) Allocation of dogs to treatment groups and groups to pens
0	Collars (IVP) fitted to IVP dogs (n = 12)
56	Allocated four of the IVP-treated dogs to a subgroup, IVP reserves
91	Collars (placebo) fitted to placebo dogs (n = 8)
98	Tick challenge: attached 30 ticks to placebo (n = 8) and IVP (n = 8) treated dogs
99	Counted ticks
100	Counted ticks
101	Counted ticks and removed (IVP dogs) or left in place (placebo dogs)
112	Tick challenge: attached 30 ticks to placebo (n = 8) and IVP (n = 8) treated dogs
113	Counted ticks
114	Counted ticks
115	Counted ticks and removed (IVP dogs) or left in place (placebo dogs)
140	Tick challenge: attached 30 ticks to placebo (n = 8) and IVP (n = 8) treated dogs
141	Counted ticks
142	Counted ticks
143	Counted and removed ticks
168	Tick challenge: attached 30 ticks to placebo (n = 8) and IVP (n = 8) treated dogs
169	Counted ticks
170	Counted ticks
171	Counted and removed ticks
196	Tick challenge: attached 30 ticks to placebo (n = 9)# and IVP (n = 12)* treated dogs
197	Counted ticks
198	Counted ticks
199	Counted ticks and removed (IVP dogs) or left in place (placebo dogs)
224	Tick challenge: attached 30 ticks to placebo (n = 7)~ and IVP (n = 8) treated dogs
225	Counted ticks
226	Counted ticks
227	Counted and removed ticks. Removed collars

^ Two IVP-treated dogs were withdrawn from the study (on Days 83 and 84) for adverse events unrelated to the test product, leaving only six dogs in the IVP-treated group for the tick challenge on Day 84, study 1

* The IVP-reserve dogs (n = 4) were included in study 2 between Days 196–199

An additional dog was included in the placebo group between Days 196–199

~ One placebo-treated dog was withdrawn from the study on Day 206 for treatment of tick paralysis, leaving only seven dogs in the placebo-treated group for the tick challenge on Day 224

treatment group randomly, initiating the sequence of treatment group allocation of the remaining dogs. Each treatment group was randomly allocated to one of two pen blocks within the kennel, and within treatment group, dogs were randomly allocated to individual pens within block. In the second study, dogs were allocated to treatment group based on social compatibility, as dogs were housed in groups (Day –7 to 91) or in pairs (Day 91–227). On Day 56, four of the IVP-treated dogs (n = 12) were randomly allocated to a subgroup, IVP reserves. On Day 91, each treatment group was randomly allocated to

Table 2 Classification of ticks according to their state of survival, attachment and engorgement*

Category	Survival status	Attachment status	Acaricidal effect
1	Live	Free, unattached	Not demonstrated
2	Live	Attached, unengorged	Not demonstrated
3	Live	Attached, engorging	Not demonstrated
4	Dead	Free, unattached	Yes
5	Dead	Attached, unengorged	Yes
6	Dead	Attached, engorging	Yes
7	Dead	Attached, engorged	Not demonstrated

* Adapted from Marchiondo et al. (2007)

one of two pen blocks within the kennel and within each treatment group, pairs of dogs were allocated to individual pens based on social compatibility. Random allocation of treatment groups and dogs was respectively achieved by blindly drawing from a hat one of two labelled coins or a ball of paper labelled with pen number.

A collar was fitted to the neck of each dog in the IVP (Seresto®; imidacloprid 10 %, flumethrin 4.5 %; Bayer Animal Health) and placebo (IVP collar, without active ingredients) groups on Day 0 (or Day 91 for the placebo dogs in study 2), leaving a gap of approximately 2.5 cm between the collar and the neck. The excess length of collar was fitted through the loops on the collar and a single mattress suture was placed to secure the free end to the underlying collar. The collars were retained for the duration of the study. Dogs were examined three times daily for any signs of ill health and treatment sites were visually inspected by the investigator at the time of each 72 h tick count.

I. holocyclus collection and infestation

Unfed, adult, female *I. holocyclus* were collected from tick habitats in the northern Rivers region of New South Wales (NSW), Australia, and stored in a dark incubator at approximately 12 °C for up to 199 days before being placed on a dog. In both studies, the dogs were infested with ticks at 14 to 28 day intervals, specifically on Days –7, 14, 42, 56, 70, 84 (study 1) and Days 98, 112, 140, 168, 196 and 224 (study 2). At each tick challenge, every dog was infested with 30 unfed, adult, female ticks, placed on the ears (3 on each), head (5), shoulders (5 on each), midline between the shoulders (5), dorsal back at the level of the last thoracic vertebra (2) and tail base (2). Each tick was manually attached to the skin of the dog and attachment verified by gently pulling on the tick to ensure insertion of its hypostome. In most cases, ticks did not move from their initial site of attachment. To avoid contaminating placebo-treated dogs with chemicals from the IVP-treated dogs, ticks were attached to all placebo dogs before the IVP-treated dogs and the investigators changed overalls between treatment groups and washed hands with a non-acaricidal soap between dogs.

Assessing efficacy

The number of ticks remaining on dogs 24, 48 and 72 (± 4) h after infestation was assessed by digital palpation and visual inspection by two or three experienced study personnel. Searching commenced in areas furthest away from the treatment site, in those areas where ticks were manually attached and extended towards the head so that the whole dog was searched. Ticks were classified into one of seven categories, as described in Table 2. The ticks were counted *in situ* and classified, but left in place at the 24 h and 48 h post infestation tick counts and removed at the 72 h count. The ticks remaining on the placebo dogs 72 h post infestation on Days 98, 112 and 196 were not removed until the following tick challenge, to re-stimulate these dogs'

Table 3 Treatment efficacy and mean number of live and dead engorged *I. holocyclus* on dogs treated with the IVP or placebo collar 24, 48 and 72 hours after artificial infestation

Day [a]	Time (h) after infestation [b]	Placebo [c]	IVP [d]	Efficacy (%)
15	24	24.7	7.4	70.2
16	48	24.1	0.9	96.4
17	72	21.7	0.1	99.3
43	24	25.2	2.1	91.6
44	48	24.3	2.6	89.4
45	72	24.6	1.5	94.0
57	24	26.9	7.4	72.7
58	48	26.6	0.9	96.8
59	72	26.4	0.1	99.7
71	24	25.8	2.9	88.9
72	48	24.6	2.1	91.6
73	72	24.5	0.8	96.6
85	24	25.9	2.4 (*6*)	90.9
86	48	25.8	1.2 (*6*)	95.2
87	72	25.0	0.0 (*6*)	100.0
99	24	26.1	4.8	81.6
100	48	26.0	2.7	89.6
101	72	23.7	0.9	96.4
113	24	26.0	7.3	71.9
114	48	25.5	1.7	93.5
115	72	22.2	0.2	99.1
141	24	23.8	8.5	64.3
142	48	22.3	6.0	73.2
143	72	19.1	4.2	77.8
169	24	19.5	3.6	81.6
170	48	18.3	1.9	89.7
171	72	15.4	0.6	95.8
197	24	21.2 (*9*)	11.5 (*12*)	46.1
198	48	19.0 (*9*)	9.9 (*12*)	48.0
199	72	16.7 (*9*)	3.3 (*12*)	79.9
225	24	24.1 (*7*)	16.3	32.2
226	48	22.7 (*7*)	14.2	37.5
227	72	20.6 (*7*)	7.1	65.5

[a] Collars (IVP or placebo) were fitted to dogs on Day 0

[b] Each dog was infested with 30 unfed, adult, female *I. holocyclus*

[c] Geometric mean number of live and dead engorged ticks (categories 1–3 and 7) on placebo-treated dogs (n = 8 dogs, except where indicated by the italicised number in brackets)

[d] Geometric mean number of live and dead engorged ticks (categories 1–3 and 7) on IVP-treated dogs (n = 8 dogs, except where indicated by the italicised number in brackets)

immunity to holocyclotoxin. Acaricidal efficacy was calculated using geometric mean numbers of ticks, as recommended by Marchiondo et al. (2007):

Acaricidal efficacy (%) = 100 x (G_{mc} – G_{mt})/G_{mc}

G_{mc} = Geometric mean number of live and dead engorged ticks (categories 1–3 and 7) on dogs in the placebo group at a specific time point.

G_{mt} = Geometric mean number of live and dead engorged ticks (categories 1–3 and 7) on dogs in the IVP group at a specific time point.

Data analysis

Descriptive statistics (mean, median, range and 95% confidence intervals (CI)) were calculated for the efficacy data using Microsoft Excel (2010).

Animal ethics and approval

Approval to conduct this study was granted by the NSW Department of Primary Industry Director General's Animal Care and Ethics Committee (09/4103) and the Australian Pesticides and Veterinary Medicines Authority General Permit PER7250.

Results

The acaricidal efficacy of the IVP against *I. holocyclus* at 72 h post infestation exceeded 95% on Days 17 (99.3%), 59 (99.7%), 73 (96.6%), 87 (100.0%), 101 (96.4%), 115 (99.1%) and 171 (95.8%), but was lower on Days 45 (94.0%) and 143 (77.8%), and declined from Day 199 (79.9%) to 227 (65.5%) (see Table 3 and Figs. 1 and 2). The mean efficacy 72 h post infestation was 97.9% over the first 115 days and 91.3% over 227 days after collar application. The mean efficacy 48 h post infestation was 93.2% over the first 115 days and 81.9% over 226 days after collar application. The mean efficacy 24 h post infestation was 81.1% over the first 115 days and 72.0% over 225 days after collar application. The mean number of live ticks counted on IVP-treated and placebo-treated dogs 72 h post infestation ranged from 0 to 7.1 and 15.4 to 26.4, respectively. As ticks were removed at 72 h post infestation, no

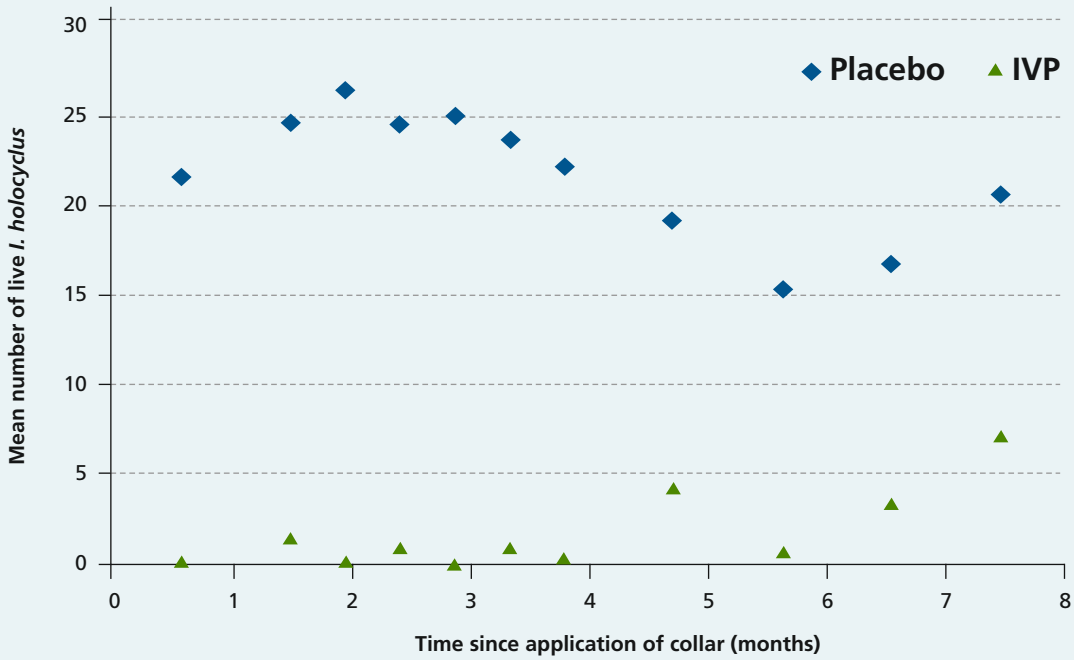

Fig. 1 Geometric mean number of live and dead engorged ticks (categories 1–3 and 7) on placebo-treated dogs (n = 7–9) or IVP-treated dogs (n = 6–12) 72 h after artificial infestation. Each dog was infested with 30 unfed, adult, female *I. holocyclus*

engorged ticks (live or dead) were seen on any IVP-treated dog. No adverse events related to treatment were observed during the study. Adverse events unrelated to treatment occurred in both IVP- and placebo-treated groups and included a swollen parotid salivary gland, gastric dilation and volvulus, tick paralysis and fight wounds and were documented and treated.

Discussion

This study has demonstrated the sustained efficacy of the IVP in dogs artificially infested with *I. holocyclus*, with mean 72 h post infestation efficacies of 97.9% over 115 days and 91.3% over 227 days after collar application. These efficacy results are similar, but not sustained for as long after collar application compared to those reported for the IVP against other tick species. For example, the efficacy of the IVP on dogs at 18 and 48 h post infestation with 50 (25 male, 25 female) laboratory-bred *R. sanguineus* exceeded 97.8% from Day 7 until the completion of the study on Day 226 (Horak et al. 2012). However, to allow for an assessment of repellency 6 h post infestation, ticks were not manually attached to the dogs in Horak's study, which likely resulted in fewer ticks attaching to the treated dogs and lower tick counts compared to the current study. Another study that similarly investigated repellent efficacy at 6 h post infestation also reported very good efficacy in dogs 48 h post infestation against *I. ricinus* (100%), *R. sanguineus* (>97%), *D. reticulatus* (generally >97%) and *D. variabilis* (>90%) from Day 2 to Day 240 after application of the IVP (Stanneck et al. 2012b). High and sustained acaricidal efficacy has also been demonstrated against naturally acquired tick infestations, with efficacies of 91.2–100% (mean 94.7%) over 8 months reported in 197 dogs treated with the IVP in a European

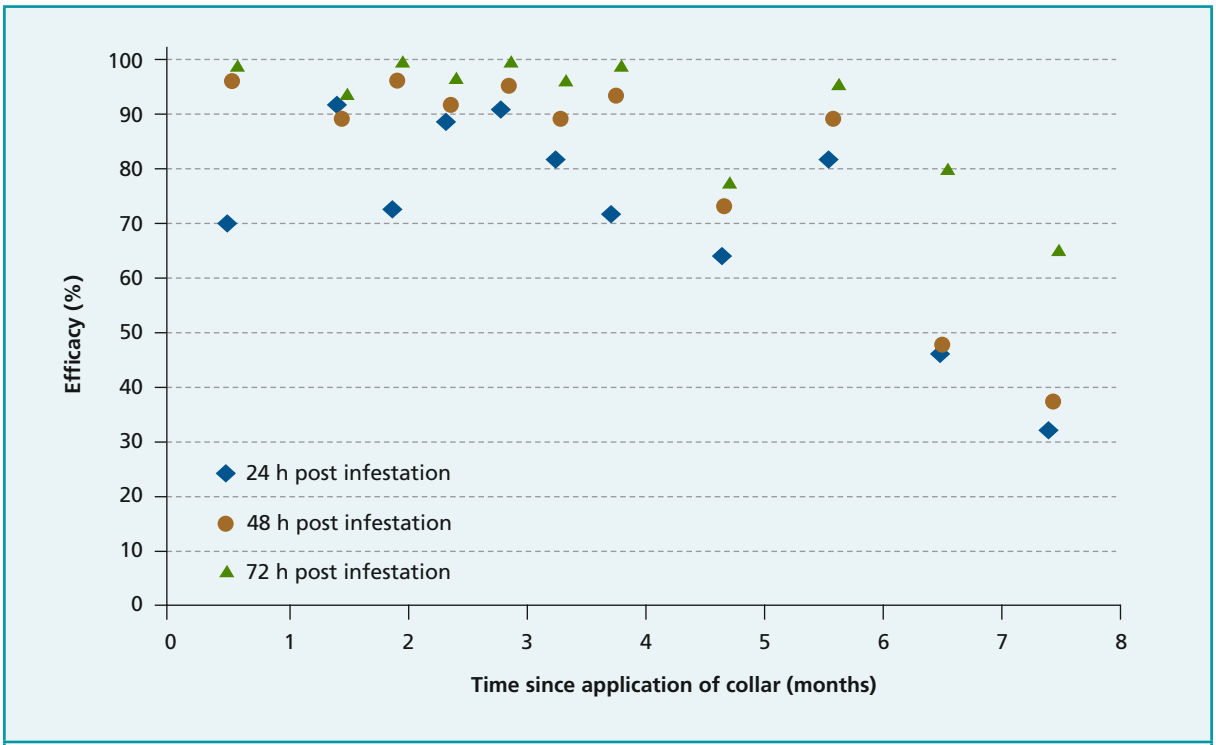

Fig. 2 Efficacy of the IVP collar against *I. holocyclus* over 8 months

multicentre clinical field study (Stanneck et al. 2012d).

The current study was designed to assess the acaricidal efficacy of the test product to support the Australian registration of the IVP for the prevention of tick paralysis induced by *I. holocyclus*. For this reason, each tick was manually attached to the dog ensuring insertion of its hypostome, a method not conducive to assessing repellency, as the ticks are not required to move through the hair to voluntarily attach to the dog. With regard to the acaricidal efficacy this test represents an artificial, worst case scenario as ticks are much more likely to acquire a lethal dose of the active ingredient while moving though the hair coat in a natural setting before finally attaching to the animal. This design gives us a clearer understanding of the acaricidal efficacy of the test product even under maximally unfavourable conditions (a tick attaching immediately to the spot of its first animal contact), whilst it disregards the repellent properties of pyrethroids that have been demonstrated for ticks in both *in vitro* (e.g. moving object bioassay) (Dautel and Cranna 2006) and *in vivo* studies (Horak et al. 2012, Stanneck et al. 2012b).

As the adult, female *I. holocyclus* feed, they inject toxins into their host that can cause fatal paralysis in dogs. It is therefore critical that ticks are killed or their ability to feed impaired prior to the onset of clinical signs of paralysis, which may be seen as early as the fourth day after attachment (Clunies Ross 1935) and on average 6.2 days post infestation (Ilkiw et al. 1987). This corresponds to findings that the quantity of toxin secreted by the ticks' salivary glands increases rapidly from the third day of feeding (Goodrich and Murray 1978) and justifies the timing of the tick counts for calculation of acaricidal efficacy in this study. A tick killed by 72 h post infestation is unlikely to have induced paralysis in its host, making this a clinically relevant and standard time to determine efficacy against *I. holocyclus* (Webster et al. 2011).

Fig. 3 *Ixodes holocyclus* fully engorged female (left: dorsal view, right: ventral view) © Anne Fawcett

An evaluation of efficacy against *I. holocyclus* on IVP-treated dogs following shampooing or swimming was beyond the scope of this current study, but has been previously assessed for other tick species. Stanneck et al. (2012b) found that the efficacy of the IVP in dogs that were shampooed or immersed in water every 28 days remained above 97% and 94%, respectively, against *R. sanguineus* and above 90% and 89%, respectively, against *D. variabilis*, over a period of 8 months. These results suggest that the active acaricidal compound remains in or on the hair and skin, or are replenished from the collar and thus support an assumption that regular shampooing or swimming should not markedly reduce the IVP's acaricidal efficacy against *I. holocyclus*.

A limitation of this study was that dogs were housed in pairs for some of the tick challenges (between Days 91 to 227), potentially allowing dogs to remove ticks from their pen-mates, reducing tick counts. It was suspected that grooming by the pen-mate (dog 9) was responsible for the declining tick counts in one placebo-treated dog (dog 67) of 17, 11, 9 and 3, 72 h post infestation on Days 101, 115, 143 and 171, respectively. To test this hypothesis, dog 62 was substituted for dog 67 and housed with dog 9 for the tick challenge commencing on Day 196 and similarly exhibited a very low tick count (3) 72 h post infestation. At the tick challenge subsequent to separation from dog 9 (Day 224), dog 62 had a higher 72 h post infestation tick count, suggesting that dog 9 had previously removed ticks by grooming her pen-mates. This occurrence may explain the declining tick counts observed in the placebo group mean tick counts from Days 98–168. There were no adverse events related to the IVP in this study, which supports the safety data already compiled through field trials and long-term safety studies in dogs and puppies. In a European field study, only 3 of 271 IVP-treated dogs exhibited treatment-related adverse events over the 8 month study period, and these were confined to minor, local reactions at the collar site (Stanneck et al. 2012d).

Conclusion

This study has demonstrated the excellent acaricidal efficacy (mean = 97.9 %) of the IVP against *I. holocyclus* 72 h post infestation over 16 weeks.

Ethical standards

Approval to conduct this study was granted by the NSW Department of Primary Industry Director General's Animal Care and Ethics Committee (09/4103) and the Australian Pesticides and Veterinary Medicines Authority General Permit PER7250.

Conflict of interest

Bayer Animal Health Australia completely funded this study and is the employer of W Smith, L Ahlstrom and R Rees. Veterinary Health Research (VHR) Pty Ltd is a privately owned, independent, contract research facility in Australia, contracted to conduct this study. Wongaburra Research Centre is a subcontractor of VHR.

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