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Efficacy and safety of Felpreva®, a spot-on formulation for cats containing emodepside, praziquantel and tigolaner against experimental infestation with the Australian paralysis tick *Ixodes holocyclus*

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ABSTRACT

The Australian paralysis tick *Ixodes holocyclus* continues to be a serious threat to companion animals along Australia's east coast. The tick produces a potent neurotoxin which causes a rapidly ascending flaccid paralysis, which if left untreated, can result in the death of the animal. There is currently only a limited number of products registered in Australia for the treatment and control of paralysis ticks in cats. Felpreva® is an effective spot-on combination containing emodepside, praziquantel and tigolaner. To investigate the therapeutic and long-term persistent efficacy of Felpreva® (2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner) against experimental infestation with *I. holocyclus* in cats, two studies were undertaken. Fifty cats were included in the studies on study Day -17. These cats were immunized against paralysis tick holocyclotoxin prior to the study commencing. Immunity to holocyclotoxin was confirmed with a tick carrying capacity (TCC) test conducted prior to treatment. Cats were treated once on Day 0. Group 1 cats were treated with the placebo formulation and Group 2 cats were treated with Felpreva®. Cats were infested on Days -14 (tick carrying capacity test), 0, 28, 56, 70, 84 and 91 (weeks 4, 8, 10, 12 and 13). Ticks were counted on cats 24 h, 48 h and 72 h post-treatment and infestation, except during the tick carrying capacity test when they were counted approximately 72 h post-infestation only. The 24-h and 48-h assessments were conducted without removing the ticks. The ticks were assessed, removed and discarded at the 72-h assessment time-points. Significant differences in total live tick counts at ~24 h, ~48 h and ~72 h post-infestation were observed between the treatment and control group. Differences were significant ($P < 0.05$ to < 0.001) in all instances. Treatment efficacies of 98.1–100% were observed ~72 h post-infestation through to 13 weeks (94 days) post-treatment. These results show that a single application of Felpreva® provides effective treatment and control against induced infestation with paralysis ticks for 13 weeks.

1. Introduction

Infestations with the Australian paralysis tick *Ixodes holocyclus* remain to be a major problem in companion animals in Australia. This tick is clinically the most significant tick species in Australia because it produces a potent neurotoxin (holocyclotoxin) that causes a rapidly ascending flaccid paralysis which can be fatal and each year thousands of cases are reported in dogs and cats (Barker & Walker, 2014; Guernier et al., 2016). Coastal areas with dense bushland and vegetation cover, combined with high humidity, temperate climates throughout most of the year and the availability of suitable bandicoot wildlife hosts create a favorable environment for the tick's survival and development. The distribution of *I. holocyclus* is limited to coastal areas of Australia's east coast and extends from southeastern Victoria, throughout New South Wales to northern Queensland and most cases of tick paralysis are reported in spring and summer (Barker & Walker, 2014).

Approximately three days following attachment, the activity and size of the tick's salivary glands increase which is associated with the release of the holocyclotoxin. The toxin interferes with the presynaptic release of acetylcholine (Chand et al., 2016) in the affected host and a single female

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I. holocyclus tick is enough to cause potential paralysis and death. Clinical signs typically start to develop after ~72 h of feeding and include altered voice, laboured respiration, ascending flaccid paralysis eventually leading to respiratory failure and death (Masina & Broady, 1999). A study that retrospectively investigated the occurrence of tick-induced paralysis in cats that were presented to four emergency clinics in Queensland between 2008 and 2016 reported a total of 2077 cases over this period. Out of these 2077 cases, 273 cats either died or had to be euthanized (Leister et al., 2018). The detection of a single infesting tick can be challenging, and attachment may occur in locations that are difficult to examine such as between the digits, inside the anus, vulva or on the hard palate. These locations are also not protected by topically acting acaricides leaving the hosts susceptible to infestation and the subsequent development of tick-induced paralysis. Therefore, significant advantages can be gained from the use of systemically acting acaricides that also provide protection on areas that are distant from the application site (Baker et al., 2018).

Felpreva® containing the cyclic depsipeptide emodepside in combination with praziquantel, provides effective control of a wide range of helminth parasites including nematodes (roundworms, hookworms and lungworms) and cestodes (Cvejić et al., 2022b; Traversa et al., 2022). Tigolaner offers protection against fleas and ticks for 13 weeks (Cvejić et al., 2022a; Mencke et al., 2023). In the present paper, we report on two efficacy studies that investigated the efficacy of Felpreva® for the control of *I. holocyclus* in cats.

2. Materials and methods

2.1. Study design and animals

Two randomised, negative-controlled, efficacy studies were conducted to determine the therapeutic and long-term persistent efficacy of Felpreva® spot-on (2.04% w/v emodepside, 8.14% w/v praziquantel and 9.79% w/v tigolaner) on cats against experimental infestations of *I. holocyclus*. The studies were carried out between April and November 2019 and were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013), Veterinary International Conference of Harmonization Guidelines (EMA, 2000), 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 Infestation on dogs and cats (Marchiondo et al., 2007) and the Australian Pesticides and Veterinary Medicines Authority (APVMA) Preamble for the WAAVP guidelines for fleas and ticks on dogs and cats (APVMA, 2014). Animals were handled in compliance with Animal Research Authority nos. BAA F 18150 W and BAA F 18186 W issued by the Wongaburra Animal Ethics Committee, and applicable local regulations.

Cats were sourced from the Wongaburra Research Centre cat colony. For each study 26–28 cats were immunized against holocyclotoxin by attaching gradually increasing numbers of ticks to the cats at weekly intervals prior to the study animal phase commencing. The ticks were left on the cats for a maximum period of 3 days. The cats were monitored at least twice daily during the immunization process. Any animal that developed clinical signs of tick paralysis was to have all ticks removed and tick anti-toxin serum given if required. Cats were acclimatized for 17 days. For both studies, domestic cats of mixed breeds (long- and short-haired) and of both sexes and neuter status were used. Cats were between 3 and 10 years of age and had a body weight of 3.5–7.7 kg at the time of study commencement (Day 0). Pre-enrolment veterinary clinical examination was conducted on Day -17 for all cats to confirm good clinical health and suitability for study participation.

Housing of cats complied with the guidelines of the Council of Europe (Cons 123, 2006; Appendix A), as required under Animal Research Establishment accreditation from the New South Wales Department of Primary Industries. Cats were housed in pens with a floor area 1.5 × 3 m, equal areas located inside and outside, that allowed each cat to see neighbouring cats through a transparent door. Cats were housed individually whilst infested with ticks. At times when cats were not infested with ticks, they shared housing with up to two other socially compatible cats within the same treatment group. There was no contact between treatment groups to prevent chemical transfer. Cats were individually housed from Day 0 to Day 5 (Study 1) or Day 6 (Study 2) to allow time for the treatments to dry. The placebo-treated Group 1 cats were tended to first during routine husbandry (e.g. feeding, cleaning) and study procedures (e.g. weighing, tick infestations and tick counts) before the treated Group 2 cats. Cats were fed once daily with a standard feline diet and water was provided *ad libitum*.

2.2. Allocation and treatment

Pre-treatment tick carrying capacity (TCC) test was conducted on Day -14 for allocation purposes and to confirm that cats were sufficiently immunized to holocyclotoxin and free of any residual acaricidal efficacy prior to treatment. On Days -10 (Study 1) or -7 (Study 2) cats were allocated to study groups based on TCC. Cats were ranked in descending order of total live ticks [TOL = Live attached (LA) + Live free (LF)] 72 h post-infestation. Twenty cats were then selected for each study; 10 for Group 1 (placebo-treated) and 10 for Group 2 (Felpreva®-treated). The next two cats were selected as spare Group 1 placebo-treated cats. Cats with the lowest tick carrying capacity were excluded. The 20 selected cats were grouped by coat length (long or short) and 10 replicates of two cats were formed. Each cat was randomly allocated to Group 1 or Group 2. Data was sorted by group and cats were paired according to compatibility (non-random) within treatment group. Pairs of cats were then randomly allocated to pairs of pens within the cattery.

Cats were weighed on Day 0 prior to treatment application. Doses were administered topically by parting the fur on the cat's neck at the base of the skull and applying the spot-on directly onto the skin. Doses corresponded to the minimum effective dose of 0.148 ml/kg body weight, for Felpreva®. Cats that met the following inclusion criteria were enrolled in the study: (i) clinically healthy, including no abnormal signs at the application site as determined by the attending veterinarian/investigator on Day -17; (ii) not clinically pregnant, not excessively fractious; (iii) between 1 and 10 years of age, > 1 kg and less than 8 kg at time of allocation; (iv) manageable and cooperative with study procedures; (v) not treated with a long-acting topical or systemic acaricide/insecticide for at least 2 months before the start of the study; and (vi) tick carrying capacity greater than that of the 2 lowest animals.

2.3. Source of ticks and cat infestation procedure

Unfed adult female *I. holocyclus* ticks, collected between August 2018 and October 2019 from at least three different localities in the Northern Rivers area of New South Wales and/or south-east Queensland, and Far North Queensland, were used in the studies (Table 1). The ticks were maintained in a dark incubator at optimal conditions of temperature and humidity prior to use.

Each cat was infested by manually attaching a total of 10 adult female ticks to the head, shoulders and mid-back. The majority of ticks were attached to the head and shoulders to simulate the tick's natural predilection for these sites. Ticks were placed at skin level and encouraged to attach by gently tapping them with a finger. When attached, they assumed a head down position with their maxillary palps spread. The hypostome (mouthpart) was not visible. Cats were infested on Days 0 (2 h prior to treatment), 28, 56, 70, 84 and 91. The thermostat in the cattery temperature control system was set to a minimum temperature of 18 °C while cats were infested with ticks. Cats were held indoors following infestation until after the 24-h tick assessments. Placebo-treated cats (Group 1 plus two spares) were infested with 4 ticks each approximately mid-way between experimental infestations (Day 15 and Day 42 for Study 1; Day 14 and Day 43 for Study 2) to maintain immunity to

Table 1
The number of sampling locations and proportions of ticks used in the studies by state.

Study	State	No. of sampling locations	No. of ticks collected per state	Percentage of total
Study 1	New South Wales	24	692	48.00
	South East Queensland	14	525	36.50
	Far North Queensland	6	223	15.50
	Total	44	1440	
Study 2	New South Wales	11	902	61.78
	South East Queensland	5	318	21.78
	Far North Queensland	5	240	16.44
	Total	21	1460	

holocyclotoxin. The two spare placebo-treated cats were infested with 4 ticks each during experimental infestations to maintain immunity to holocyclotoxin on Days 0, 28, 56, 70 and 84. The ticks were removed after 3 days.

2.4. Health observations

The health status of cats was monitored daily during the immunization and acclimatization period and for the entire duration of the study. Cats were subjected to a thorough veterinary examination to confirm suitability for inclusion in the study on Day -17 and were then monitored 3 times daily for general health until study completion. Particular attention was paid to symptoms of tick paralysis including incoordination, hind limb paralysis, paresis, pupillary dilation, reduced appetite, changes in vocalisation, dyspnea and respiratory compromise. Each cat was held for 1 min following treatment administration then observed for 5 min for general behaviour. Clinical observations were made on all cats prior to treatment, at approximately 1 h, 24 h and 48 h following treatment of the last animal. Clinical observations were also performed on Days 7, 28, 56 and 84.

2.5. Parasitological examinations

For the TCC test, ticks were counted and removed on Day -11 (approximately 72 h post-infestation). Tick safety searches were conducted approximately 96 h post-infestation. Ticks were counted on cats 24 h, 48 h and 72 h post-treatment and post subsequent infestations (Table 2). The 24-h and 48-h assessments were carried out without removing the ticks. Ticks were assessed, removed and discarded at the 72-h tick counts. Tick safety searches were conducted approximately 96 h post-infestation. Tick safety searches were a precaution to reduce the risk of potential tick paralysis from stray or missed ticks. Any ticks identified during the tick safety searches were removed and discarded. The tick safety searches were not time-dependant and ticks found at this timepoint were not included in the assessment of efficacy. Ticks were counted on cats of one study group at a time, to reduce the potential for chemical transfer between groups. The attachment locations used during the experimental infestations were inspected first, followed by a full body search. Ticks were located by digital palpation. In areas of sparse or short hair (e.g. inner ears, lips and groin) the ticks were located by visual inspection.

Ticks were classified according to viability (live or dead) and attachment status. Attached ticks (A) had their hypostome embedded into the skin of the cat and were not easily dislodged from the cat. Free ticks (F) were unattached ticks. They may have been live and moving through the coat, or dead and sitting in the hair. The ticks found on the cats were assessed using the parameters outlined in Table 3.

Classification was a subjective process undertaken by a suitably experienced tick assessor. Live (L) ticks demonstrated active leg movement, normal engorgement and no crenation. Inflammation and exudate (oozing serum) may have been observed around the attachment site. Tick

Table 2
Detailed study schedule for Study 1. Critical activities that were performed to determine the acaricidal efficacy of Felpreva® are highlighted in bold.

Study day	Activity
Pre-study	Immunization of cats
-17	Veterinary examinations all study cats
	Commence three times daily monitoring
-16 to -15	Monitor cats
-14	TCC infest cats
-13 to -12	Monitor cats
-11	TCC count and remove ticks
-10	96-h tick safety search
	Allocate
-9 to -8	Monitor cats
-7	Re-pen cats
-6 to -1	Monitor cats
0	**Weigh each cat prior to infestation**
	Infest cats with 10 ticks each (including spare placebo treated cats with 4 ticks each)
	Pre-treatment clinical observations
	Treat. Hold cats for 1 min post-treatment administration
	Observe each cat for 5 min post-treatment
	1-h post-treatment clinical observations
1	24-h post-treatment clinical observations
	24-h post-treatment tick assessment
2	48-h post-treatment clinical observations
	48-h post-treatment tick assessment
3	**72-h post-treatment tick assessment and remove**
4	96-h tick safety search
5 to 6	Monitor cats
7	Clinical observations
	Deworm placebo treated cats
8 to 14	Monitor cats
15	Infest placebo treated cats with 4 ticks each to maintain immunity
16 to 17	Monitor cats
18	Remove immunising ticks
19	96-h tick safety search
20 to 27	Monitor cats
28	Clinical observations
	Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)
29	**24-h post-infestation tick assessment**
30	**48-h post-infestation tick assessment**
31	**72-h post-infestation tick assessment and remove**
32	96-h tick safety search
33 to 41	Monitor cats
42	Infest placebo treated cats with 4 ticks each to maintain immunity
43 to 44	Monitor cats
45	Remove immunising ticks
46	96-h tick safety search
47 to 55	Monitor cats
56	Clinical observations
	Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)
57	**24-h post-infestation tick assessment**
58	**48-h post-infestation tick assessment**
59	**72-h post-infestation tick assessment and remove**
60	96-h tick safety search
61 to 69	Monitor cats
70	**Infest cats with 10 ticks each (including spare placebo-treated cats with 4 ticks each)**
71	**24-h post-infestation tick assessment**
72	**48-h post-infestation tick assessment**
73	**72-h post-infestation tick assessment and remove**
74	96-h tick safety search
75 to 83	Monitor cats
84	Clinical observations
	Infest cats with 10 ticks each (including spare placebo treated cats with 4 ticks each)
85	**24-h post-infestation tick assessment**
86	**48-h post-infestation tick assessment**
87	**72-h post-infestation tick assessment and remove**
88	96-h tick safety search
89 to 90	Monitor cats
91	**Infest cats with 10 ticks each**

(*continued on next page*)

Table 2 (*continued*)

Study day	Activity
92	**24-h post-infestation tick assessment**
93	**48-h post-infestation tick assessment**
94	**72-h post-infestation tick assessment and remove**
95	96-h tick safety search

Table 3
Tick classification according to viability and attachment status. Adapted from Marchiondo et al. (2013).

Survival status	Attachment status	Abbreviation	Interpretation
Live	Free	LF	Acaricidal effect not demonstrated
Live	Attached	LA	Acaricidal effect not demonstrated
Dead	Free	DF	Acaricidal effect demonstrated
Dead	Attached	DA	Acaricidal effect demonstrated

Abbreviations: L, live; F, free; D, dead; A, attached.

faeces may also have been present. Dead (D) ticks showed no leg movement, did not react when stimulated, and may have appeared crenated or desiccated. Moribund (M) ticks were those that were classified as being dead on the cat, but then displayed feeble leg movement after removal from the cat. Moribund ticks were recorded as LA but were noted to be moribund. Moribund ticks are considered incapable of causing tick paralysis but were included in the live tick count as per APVMA requirements. The total live count (TOL) consisted of all live ticks found on a cat.

2.6. Efficacy assessments and statistical methods

The total number of live ticks (TOL) was used in the calculation of efficacy. Efficacy was calculated based on arithmetic and geometric mean TOL. Treatment effects for 'Efficacy' were calculated in all instances using TOL tick count counts 24 h, 48 h and 72 h post-infestation and the formula: Treatment effect (%) = (Mean Placebo count – Mean Felpreva count)/Mean Placebo count.

All health and clinical observations/examinations were evaluated clinically but were not statistically analysed.

3. Results

3.1. Inclusion criteria, health observations and safety assessments

Out of 24 (Study 1) and 26 (Study 2) cats screened during pre-enrolment veterinary examination and TCC, 20 cats were enrolled in the study based on highest tick counts. For each study, two cats were selected as spares and cats with the lowest tick counts were excluded from the study. There were four adverse events recorded during each of the two studies which were mild in nature and unrelated to the treatment with Felpreva®. Recorded adverse events included sneezing and nasal discharge, swelling on the forehead or of the eye, areas of alopecia, moist dermatitis and skin reddening. These adverse events were associated with tick attachments and usually resolved without any intervention.

3.2. Statistical analysis

A preliminary data exploration was conducted prior to statistical analyses; summary statistics of tick counts and bodyweights prior to treatment. Pre-treatment TOL tick counts appeared to be approximately normally distributed within the overall group of selected cats, with similar median and mean values. When standard deviations were expressed as a percentage of the group mean (coefficient of variation) they were 25–32% (Study 1) or 31–35% (Study 2), indicating relatively moderate variability in the data. Homogeneity of variances for untransformed and log-transformed TOL 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, ANOVA) for comparison of group means. Log-transformation of the data appeared to offer an advantage relative to untransformed (raw) data according to Levene's test results and a slightly improved Shapiro-Wilks normality test *P*-value, hence TOL counts were log-transformed for statistical comparisons. Parametric ANOVA was used to compare TOL counts at allocation and bodyweights prior to treatment, using fixed-effects linear models and the statistical package Spotfire S + Version 8.2, Tibco Software Inc. 2010:

$$TOL.Allocation \sim Treatment + Coat + Sex + Age$$

$$Weight.Day0 \sim Treatment + Replicate + Coat + Sex + Age$$

Post-treatment TOL tick counts were compared using the same package and the fixed-effects linear model:

$$(Count) \sim Treatment + Replicate + Weight.Day0 + Coat + Sex + Age$$

Group mean tick counts were compared at a family-wise significance level of $P < 0.05$ using Tukey's multiple comparison test, with results of pairwise group comparisons presented as confidence intervals. Residuals output was generally acceptable and terms in the model tended to be non-significant with the exception of *Treatment.Group*. Means, medians, standard deviations and coefficients of variation were calculated to assess the normality (or otherwise) of study data.

3.3. Acaricidal efficacy

For both studies, TOL tick counts prior to treatment were similar for both groups, with no significant differences observed at $P < 0.05$. Treatment groups could therefore be considered equivalent prior to treatment. Post-treatment, mean tick counts in placebo-treated cats ranged from 4.9 to 7.7 (mean = 6.3; standard deviation, SD = 0.76) in Study 1 and from 5.2 to 7.0 (mean = 6.1, SD = 0.55) in Study 2 at each sampling time-point and showed that tick infestation was adequate on placebo-treated cats and that trial results can be used to determine treatment efficacy (Table 4). The mean tick counts in Felpreva®-treated cats ranged from 0 to 4.6 (mean = 0.6, SD = 1.06) in Study 1 and from 0 to 3.1 (mean = 0.4, SD = 0.70) in Study 2, and were significantly lower compared to mean tick counts on placebo-treated cats and for each time-point post-treatment or post-infestation. For Study 1, highly significant differences in TOL tick counts were observed between the two groups at ~24 h ($P < 0.05$ to < 0.001), ~48 h ($P < 0.001$) and ~72 h ($P < 0.001$) at all time-points during the study. For Study 2, highly significant differences in TOL tick counts were observed between the two groups at ~24 h ($P < 0.001$) apart from the first occasion (Day 1, $P = 0.038$, however, confidence intervals spanned zero), ~48 h ($P < 0.001$) and ~72 h ($P < 0.001$) at all time-points during the study.

Across the two studies, the acaricidal efficacy of Felpreva® was between 37.0% (Study 1, Day 1) and 98.6% (Study 2, Day 29) at 24 h and between 89.1% (Study 1, Day 2) and 100% (Study 1, Day 30) at 48 h. In Study 1, the acaricidal efficacy of Felpreva® against *I. holocyclus* at the 72-h assessment reached 100% on Days 3, 31, 59, 73, was 98.2% on Day 87, and again reached 100% on Day 94. In Study 2, the acaricidal efficacy of Felpreva® at the 72-h assessments reached 100% on Days 3, 31, 59, 87, and 98.1% on Days 73 and 94 (Table 4). The efficacy of Felpreva® was > 95% (range 98.1–100%) at all 72-h time-points during both studies. This is within the critical period before toxin production takes place and clinical signs of tick paralysis start to develop.

Table 4
The treatment efficacies based on the arithmetic mean Ixodes holocyclus tick counts in Felpreva® and placebo-treated cats at 24 h, 48 h and 72 h following experimental infestation.

Time following treatment (days)	Time after treatment or reinfestation (hours)	Study 1			Study 2		
		Placebo	Felpreva®	Efficacy (%)	Placebo	Felpreva®	Efficacy (%)
1	24	7.3	4.6	37.0	6.1	3.1	49.2
2	48	6.4	0.7	89.1	5.7	0.1	98.2
3	72	5.7	0	100	5.4	0	100
29	24	6.3	0.1	98.4	6.9	0.1	98.6
30	48	5.9	0	100	6.7	0.1	98.5
31	72	5.3	0	100	6.4	0	100
57	24	7.4	0.8	89.2	7.0	0.2	97.1
58	48	6.4	0	100	6.3	0.2	96.8
59	72	6.2	0	100	5.8	0	100
71	24	7.0	0.7	90.0	6.6	0.8	87.9
72	48	6.2	0	100	5.7	0.5	91.2
73	72	5.6	0	100	5.2	0.1	98.1
85	24	7.7	1.2	84.4	6.8	0.3	95.6
86	48	6.8	0.2	97.1	5.9	0	100
87	72	5.7	0.1	98.2	5.5	0	100
92	24	7.1	1.2	83.1	6.4	0.6	90.6
93	48	5.6	0.4	92.9	5.7	0.2	96.5
94	72	4.9	0	100	5.4	0.1	98.1

4. Discussion

The effective control of ectoparasites (ticks and fleas) in cats is of major importance in veterinary practice as well as for pet owners. Both, ticks as well as fleas are known vectors for a variety of pathogens causing vector-borne disease in companion animals. In addition to infections with bacterial, viral or protozoon pathogens, infestations with the Australian paralysis tick can cause life-threatening paralysis and if untreated, result in the death of the animal. Treatment of affected cats often requires intensive emergency critical care and hospitalisation and can be very costly to the owner. *Ixodes holocylus* is not the only tick capable of producing a potent toxin and cases of tick paralysis have also been reported from other continents, as for example in Europe, where mortalities of dogs have been reported as a result of tick paralysis induced by *Rhipicephalus sanguineus* (Otranto et al., 2012). Therefore, the best approach to the control of this parasite is the treatment with effective acaricides that kill the ticks before they release their toxins *via* saliva and thus before clinical signs of paralysis occur. Currently, there is only a limited number of registered products in Australia that offer effective control of *I. holocyclus* in cats. Topically distributed acaricides available for cats are available as sprays, shampoos or collars. Sprays and shampoos, depending on the active substances and concentrations only provide control for a limited period of time (3 days–3 weeks) and require frequent reapplication which represents a challenge to owner compliance and increases the likelihood of cats being exposed to the parasite if treatment intervals are not stringently followed. There is currently only one collar containing flumethrin and imidacloprid registered in Australia for cats which repels and controls paralysis ticks for up to 8 months. However, collars can also be easily lost and topically acting and distributed acaricides may have limited effect on ticks that attach in obscure locations. Therefore, systemically acting acaricides that are topically applied provide an effective and easy-to-use approach for the control of paralysis ticks in cats. Also, products that offer a longer duration of protection have been suggested to increase owner compliance as less frequent reapplication of the treatment is required (Lavan et al., 2017). At present, there are only four such registered products available for cats in Australia which provide protection for 5 weeks to 3 months (reviewed by Roeber & Webster, 2021). Two of these products also contain macrocyclic lactones for added treatment of nematode infections but none of these products contains an active for the treatment of tapeworms. Felpreva® is the first combination product for cats that can be topically applied but is systemically distributed and offers long-lasting (up to three months) protection against paralysis ticks and also contains actives for the control of nematodes and cestodes (emodepside and praziquantel). The efficacy of Felpreva® against experimental *I. holocyclus* infestations was > 95% at all 72-h time-points during both studies which confirms that Felpreva® is effective in the treatment of *I. holocyclus* infestations and kills ticks before clinical signs of tick paralysis can develop. However, regardless of high efficacy achieved, no product will be fully effective on all occasions and an effective *I. holocyclus* protective strategy will also require owners and veterinarians to remain vigilant and ensure that treatment intervals are followed, and regular tick searches are being conducted in animals living in high-risk areas.

There were four adverse events reported during both studies which were mild in nature and related to tick attachment reactions. There were no adverse reactions observed to the treatment with Felpreva® itself, confirming that the treatments were well tolerated in all animals.

5. Conclusions

In conclusion, the combination of a highly effective systemically acting bispyrazole acaricide together with the endoparasiticides emodepside and praziquantel, Felpreva® represents a convenient all-in-one solution for the treatment and control of ecto- and endoparasites in cats. Efficacy of > 95% was demonstrated for three months following treatment which provides an extended time of protection and reduces the number of re-applications, thus increasing owner compliance.

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Ethical approval

The studies were carried out between April and November 2019 and were conducted in compliance with the Australian Code for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Housing of cats complied with the guidelines of the Council of Europe (Cons 123, 2006; Appendix A), as required under Animal Research Establishment accreditation from the New South Wales Department of Primary Industries. Animals were handled in compliance with Animal Research Authority nos. BAA F 18150 W and BAA F 18186 W issued by the Wongaburra Animal Ethics Committee, and applicable local regulations.

CRediT authorship contribution statement

Florian Roeber: Investigation, Methodology, Resources, Supervision, Writing – original draft. **Chrissie Jackson:** Investigation, Methodology, Resources, Supervision. **Michael Chambers:** Data curation. **Veronica Smith:** Conceptualization, Methodology, Project administration. **Jane Hume:** Writing – review & editing. **Katrin Blazejak:** Writing – review & editing. **Norbert Mencke:** Conceptualization, Funding acquisition, Writing – review & editing. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Florian Roeber, Chrissie Jackson and Michael Chambers are employees of Invetus Pty Ltd. Veronica Smith is an employee of Animal Ethics Pty Ltd. Jane Hume is an employee of Vetoquinol Australia. Katrin Blazejak and Norbert Mencke are employees of Vetoquinol S.A.

Data availability

The data supporting the conclusions of this article are included within the article. Raw data generated during the study are confidential.

References

APVMA, 2014. Preamble for the WAAVP guideline for fleas and ticks on dogs and cats. Australian Pesticides and Veterinary Medicines Authority, Canberra. https://apvma.gov.au/node/1040 (Accessed 5 January 2023).

Baker, K., Ellenberger, C., Murphy, M., Cavalleri, D., Seewald, W., Drake, J., Nanchen, S., Hacket, K., 2018. Laboratory evaluations of the 3-month efficacy of oral lotilaner (Credelio™) against experimental infestations of dogs with the Australian paralysis tick, *Ixodes holocyclus*. Parasites Vectors 11, 487. https://doi.org/10.1186/s13071-018-3061-8.

Barker, S.C., Walker, A.R., 2014. Ticks of Australia. The species that infest domestic animals and humans. Zootaxa 3816, 1–144. https://doi.org/10.11646/zootaxa.3816.1.1.

Chand, K.K., Lee, K.M., Lavidis, N.A., Rodriguez-Valle, M., Ijaz, H., Koehbach, J., et al., 2016. Tick holocyclotoxins trigger host paralysis by presynaptic inhibition. Sci. Rep. 6, 29446. https://doi.org/10.1038/srep29446.

Cons 123, 2006. Appendix A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123). Council of Europe, Strasbourg. https://www.coe.int/t/e/legal_affairs/legal_co-operation/biological_safety_and_use_of_animals/laboratory_animals/2006/Cons123(2006)3AppendixA_en.pdf.

Cvejić, D., Hellmann, K., Petry, G., Ringeisen, H., Hamburg, H., Farkas, R., et al., 2022a. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing emodepside, praziquantel and tigolaner, in treating cats naturally infested with fleas and/or ticks. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100099. https://doi.org/10.1016/j.crpvbd.2022.100099.

Cvejić, D., Mencke, N., Petry, G., Ringeisen, H., Hamburg, H., Hellmann, K., et al., 2022b. Multicenter randomized, and blinded European field study evaluating the efficacy and safety of Felpreva®, a novel spot-on formulation containing tigolaner, emodepside and praziquantel, in treating cats with mixed infection with intestinal nematodes, cestodes and/or lungworms. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100098. https://doi.org/10.1016/j.crpvbd.2022.100098.

EMA, 2000. VICH GL9 Good Clinical Practices, July 2000. In: European Medicines Agency, Committee for Medicinal Products for Veterinary Use. https://www.ema.europa.eu/en/documents/scientific-guideline/vich-gl9-good-clinical-practices-step-7_en.pdf.

Guernier, V., Milinovich, G.J., Bezerra Santos, M.A., Haworth, M., Coleman, G., Soares Magalhaes, R.J., 2016. Use of big data in the surveillance of veterinary diseases: Early detection of tick paralysis in companion animals. Parasites Vectors 9, 303. https://doi.org/10.1186/s13071-016-1590-6.

Lavan, R.P., Tunceli, K., Zhang, D., Normile, D., Armstrong, R., 2017. Assessment of dog owner adherence to veterinarians' flea and tick prevention recommendations in the United States using a cross-sectional survey. Parasites Vectors 10, 284. https://doi.org/10.1186/s13071-017-2217-2.

Leister, E., Morton, J., Atwell, R., Webster, R., 2018. Clinical presentations, treatments and risk factors for mortality in cats with tick paralysis caused by *Ixodes holocyclus*: 2077 cases (2008–2016). J. Feline Med. Surg. 20, 465–478. https://doi.org/10.1177/1098612X17733628.

Marchiondo, A.A., Holdsworth, P.A., Fourie, L.J., Rugg, D., Hellmann, K., Snyder, D.E., Dryden, M.W., 2013. World Association for the advancement of veterinary parasitology (W.A.A.V.P.) second edition: guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infestations on dogs and cats. Vet. Parasitol. 194, 84–97. https://doi.org/10.1016/j.vetpar.2013.02.003.

Marchiondo, A.A., Holdsworth, P.A., Green, P., Blagburn, B.L., Jacobs, D.E., 2007. 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 infestation on dogs and cats. Vet. Parasitol. 145, 332–344. https://doi.org/10.1016/j.vetpar.2006.10.028.

Masina, S., Broady, K.W., 1999. Tick paralysis: development of a vaccine. Int. J. Parasitol. 29, 535–541. https://doi.org/10.1016/S0020-7519(99)00006-5.

Mencke, N., Blazejak, K., Petry, G., Hamburg, H., Ringeisen, H., Knoppe, T.N., et al., 2023. Immediate and long-term efficacy of Felpreva®, a new spot-on formulation containing tigolaner, emodepside and praziquantel applied as a single application to cats artificially infested with the cat flea *Ctenocephalides felis*. Curr. Res. Parasitol. Vector-Borne Dis. 3, 100122. https://doi.org/10.1016/j.crpvbd.2023.100122.

NHMRC, 2013. Australian code for the care and use of animals for scientific purposes, 8th ed. National Health and Medical Research Council, Canberra. https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes. (Accessed 5 January 2023).

Otranto, D., Dantas-Torres, F., Tarallo, V.D., Ramos, R.A.N., Stanneck, D., Baneth, G., Caprariis, D., 2012. Apparent tick paralysis by *Rhipicephalus sanguineus* (Acari: Ixodidae) in dogs. Vet. Parasitol. 188, 325–329. https://doi.org/10.1016/j.vetpar.2012.04.005.

Roeber, F., Webster, M., 2021. Protecting dogs and cats against the Australian paralysis tick, *Ixodes holocyclus* (Acari: Ixodidae): A review of the Australian acaricide registration process. Curr. Res. Parasitol. Vector-Borne Dis. 1, 100054. https://doi.org/10.1016/j.crpvbd.2021.100054.

Traversa, D., Morelli, S., Di Cesare, A., Strube, C., Raue, K., Bisterfeld, K., et al., 2022. Efficacy of two topical combinations containing emodepside plus praziquantel, and emodepside plus praziquantel plus tigolaner, for the treatment of troglostrongylosis in experimentally infected cats. Curr. Res. Parasitol. Vector-Borne Dis. 2, 100097. https://doi.org/10.1016/j.crpvbd.2022.100097.