The Efficacy of Pyriprole Topical Solution Against Infestations of Adult Ticks (Ixodes scapularis, Amblyomma americanum and Dermacentor variabilis on Dogs

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ABSTRACT

In three separate studies, the efficacy of a single treatment with a 12.5% pyriprole spot-on solution (Prac-tic®) was investigated against induced infestation with I. scapularis, A. americanum, or D. variabilis on dogs. For each tick species, one group of eight dogs treated with a placebo solution was compared with another group treated once with the 12.5% pyriprole spot-on solution at a dose rate of at least 12.5 mg/kg. The dogs were infested with 50 unfed adult ticks of the respective species at various time points during the 4 weeks that followed treatment and the surviving ticks were recorded 48 and 72 hours after re-infestation. For each tick species, efficacy was assessed for each time-point and cumulatively for the whole evaluation period. The dogs were submitted to general health observations and clinical assessments during the study. Cumulative efficacy for the whole evaluation period was >98% against I. scapularis, and >99.0% against A. americanum and D. variabilis. The product was well tolerated by the animals.

INTRODUCTION

Tick infestation of dogs is an increasing concern for many dog owners around the globe. Ticks cause not only discomfort and stress to their hosts, but are also known to transmit a number of serious diseases both to dogs and humans.[1,2] There are numerous commercial products available for protecting domestic animals from ticks.[3] However, the number

of really innovative active ingredients with strong acaricidal efficacy introduced during the last two decades is limited. Pyriprole is a new phenylpyrazole compound with potent activity against fleas and a variety of tick species.[4-6] Such an innovative active ingredient could play a significant role in the new, more complex strategies that are being discussed to protect domestic animals from ticks and other ectoparasiticides.[7-9] The present investigation was carried out to confirm the suitability of pyriprole in the form of a 12.5% spot-on solution (Prac-tic®) for the prevention and control of dog infestations with I. scapularis, A. americanum, and D. variabilis, three of the most common tick species infesting dogs in North America.

MATERIALS AND METHODS

In three separate studies the efficacy of a ready-to-use spot-on solution (Prac-tic®) containing 12.5% pyriprole against induced infestations with three tick species was investigated under controlled laboratory conditions.

Study 1 (I. scapularis)

For this study, 24 mix breed adult dogs found healthy after examination by a veterinarian were put on acclimatization on Study Day10. None had been treated with an acaricide during the 6 months that preceded the trial. On Study Day4, all the animals were submitted to a pre-test tick infestation in order to evaluate their aptness for tick infestation and the vitality of the tick strain. I. scapularis ticks were obtained from a laboratory colony. Before being used for infestation, the ticks were maintained in an incubator at 19-22°C.

All the dogs were infested with 50 ±1 unfed I. scapularis ticks/dog. Dogs were placed on a table and the ticks were equally distributed on the back line of each dog. Tick attachment was evaluated on Study Day 2 within 46 to 48 hours after infestation. Each dog was put on a table, and the ticks were counted by passing the hand through the dog's coat against its lay. All dogs showed a tick recovery rate >25%. The 16 dogs with the highest tick holding capacity were selected to be included in the trial (eight males and eight females, 2.9 to 9.8 years old, 11.3 to 31.6 kg bodyweight). On Study Day 1, the 16 animals were allocated to two treatment groups; Group 1 to be treated with a placebo and Group 2 to be treated with the medicated spot-on solution. For allocation to the treatment groups, the dogs were blocked by gender and ranked by weight. Within each gender, animals were allocated by random to Groups 1 (placebo) or 2 (medicated).

On Study Day 0, all the animals were treated with the placebo (mineral oil USP) or the medicated spot-

Table 1. Number of live I. scapularis ticks found in dogs at several time-points after treatment with a 12.5% pyriprole spot-on solution or a placebo.

Evaluation 48 hours after reinfestation								
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)				
Days after treatment	Ticks counted	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control	
5	143	15.34	11.9	0	0	0	100.00	*
13	123	14.05	6.7	0	0	0	100.00	*
21	150	17.31	7.7	7	0.49	1.7	97.17	*
29	138	14.72	8.5	0	0	0	100.00	*
Cum	554	15.14	8.6	7	0.05	0.7	99.67	*
Evaluation 72 hours after re-infestation								
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)				
Days after treatment	Ticks counted	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control	
6	156	15.98	13.3	2	0.15	0.7	99.06	*
14	155	18.18	7.3	1	0.09	0.4	99.50	*
22	142	17.85	7.1	3	0.25	0.7	98.60	*
30	150	16.09	10.8	6	0.57	0.9	96.46	*
Cum	603	15.84	9.45	12	0.25	0.7	98.42	*

* $p < 0.0001$

on containing 12.5% pyriprole (Prac-tic®, Novartis AH Inc, Basel, Switzerland) at a minimum dose level of 12.5 mg/kg according to the following dose band recommended on the product label: 1.1 ml solution for bodyweights ranging from >4.5 to 11 kg; 2.2 ml solution for dogs weighing >11 to 22 kg; and 3.3 ml solution for >22 kg bodyweight. No other acaricidal product was used during the study. Dogs were housed individually and received water ad libitum and a fixed amount of commercial dog food daily. Re-infestation of all animals with 50 I. scapularis ticks was carried out on Study Days 3, 11, 19, and 27 as previously described. Attached and unattached live ticks were counted by hand 48 hours after re-infestation on Study Days 5, 13, 21, and 29, but left on the animals. One day later, ie, 72 hours after re-infestation on Study Days 6, 14, 22, and 30, the ticks were counted again by thoroughly combing the entire coat with a fine-tooth comb. Separate combs were used for each treatment groups. After counting, all ticks were removed from the animals and discarded.

The efficacy assessment was based on the number of live ticks, attached or unattached, found on each animal after infestation. Percentage efficacy for the treated group at each considered time point and cumulatively was calculated as follows: percent efficacy = 100 × (C – T) / C, where C is the geometric mean of live ticks in the untreated control group and T is the geometric mean of live ticks in the treatment group. Descriptive statistics (mean and standard deviation) were calculated for all variables at every time-point and cumulatively. An analysis of variance using ln(count+1) was performed between each treatment group and their respective placebo control. The results were analyzed to test if the medicated treatment with the 12.5% pyriprole spot-on solution was effective in killing adult ticks at every time-point and cumulatively. The results from all analysis were tested at an alpha level of 5% ($p \leq 0.05$).

Table 2. Number of live A. americanum ticks found in dogs at several time-points after treatment with a 12.5% pyriprole spot-on solution or a placebo.

Evaluation 48 hours after re-infestation								
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)				
Days after treatment	Ticks counted	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control	
13	84	8.98	6.2	0	0.00	0.0	100.00	*
21	154	18.18	7.7	1	0.09	0.4	99.50	*
29	151	17.95	7.2	0	0.00	0.0	100.00	*
Cum	389	14.00	7.9	1	0.03	0.2	99.79	*
Evaluation 72 hours after re-infestation								
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)				
Days after treatment	Ticks counted	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control	
14	93	8.82	8.2	0	0.0	0.0	100.00	*
22	136	16.21	5.5	0	0.0	0.0	100.00	*
30	165	19.06	8.7	0	0.0	0.0	100.00	*
Cum	394	13.97	8.2	0	0.0	0.0	100.00	*

* $p < 0.0001$

All dogs were observed approximately every hour for six hours after treatment, and then at 8, 10, 12, 18, and 24 hours after treatment for evidence of adverse signs. Daily observation of health status continued throughout the study. Throughout the trial, each animal was regularly inspected for overall physical appearance behavior, salivation, pupil constriction, dermal reaction, alopecia, nervous signs, mucous mebranes, and feces, when present.

Study 2 (A. americanum)

Sixteen mongrel dogs from the research facility colony (seven males and nine females, 15 to 23 months old and 12.3 to 25.7 kg bodyweight) found healthy after inspection by a veterinarian were included in the trial. None had been treated with an acaricide during the 6 months that preceded the trial. They had been selected out of a total of 24 dogs after a pre-test to evaluate their aptness for tick infestation and the vitality of the tick strain performed as described for Study 1.

A. americanum ticks were obtained from a laboratory colony. Pre-study acclimatization, infestation procedure, housing, allocation to treatment Groups 1 (placebo) and 2 (treated with the medicated spot-on solution), and treatment on Study Day 0

were performed as described for Study 1. Re-infestations with ticks on Study Days 4, 11, 18, and 27; tick counting on Study Days 6, 13, 20, and 29 (48 hours after re-infestation); assessment of the efficacy; and statistical analysis were carried out as described for Study 1. The assessment of tick numbers 72 hours after re-infestation (on Study Days 7, 14, 21, and 30) was done first by hand. The number of ticks was recorded, and the ticks were detached with special tweezers and discarded. Immediately afterwards, the entire coat of the animals was thoroughly combed with a fine-tooth comb, and additional live ticks were counted and their number recorded. Subsequently, they were detached with special tweezers and discarded. Separate combs were used for each treatment groups.

Observation of the dogs for adverse drug reactions to treatment and general health monitoring were performed as described for Study 1.

Study 3 (D. variabilis)

Sixteen mongrel dogs from the research facility colony (eight males and eight females, 6 to 12 months old and 13.5 to 21.3 kg bodyweight) were used for this study. None had been treated with an acaricide during the 6 months that preceded the trial. They had been selected out of a total of 24 dogs after a pre-test to evaluate their aptness for tick infestation and the vitality of the tick strain performed as described for Study 1. D. variabilis ticks were obtained from a laboratory colony. Pre-study acclimatization, infestation procedure of the dogs with ticks, housing, allocation to treatment Groups 1 (placebo) and 2 (treated with the medicated spot-on solution), and treatment on Study Day 0 were performed as described for Study 1.

Re-infestations with ticks on Study Days 11, 19, and 27; tick counting on Study Days 13, 21, and 29 (48 hours after re-infestation); assessment of the efficacy; statistical analysis; observation of the dogs for adverse drug reactions to treatment; and general health monitoring were performed as described for Study 1. Tick counting on Study Days 14, 21, and 30 (72 hours after re-infestation) was carried out as described for Study 2. However, problems with procedural issues at the laboratory resulted in the invalidation of all the tick counts in the placebo-treated group assessed 72 hours after re-infestation. The percentage efficacy was calculated using the number of ticks counted 48 hours after re-infestation.

In all three studies, the personnel carrying out the clinical assessments, tick infestations, and counting were blinded regarding the dispositions of the treatments. Only the individuals involved in the allocation of animals to groups and test item administration knew the blinding schedule and were not involved in any post dosing assessments. The blinding code was not broken during the study and the group allocation was only disclosed after data collection was completed. All three studies were conducted in accordance with standard GLP guidelines applicable in the USA.

RESULTS

Study 1 (I. scapularis)

After treatment, the tick recovery rates for Group 1 (placebo) ranged between 30.8% (Study Day 13) and 39.0% (Study Day 6). In the medicated group, a cumulative total of seven live ticks were recorded 48 hours after re-infestation, resulting in a cumulative

Table 3. Number of live D. variabilis ticks found in dogs at several time-points and after treatment with a 12.5% pyriprole spot-on solution or a placebo.

Evaluation 48 hours after re-infestation							
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)			
Days after treatment	Ticks counted	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control
6	244	26.83	10.3	1	0.09	0.4	99.66 *
13	279	33.73	9.5	1	0.09	0.4	99.73 *
20	244	29.98	5.7	1	0.09	0.4	99.70 *
29	217	26.76	4.8	16	1.25	2.7	95.33 *
Cum	984	29.81	8.0	19	0.31	1.5	98.96 *
* $p < 0.0001$							
Evaluation 72 hours after re-infestation							
	Group 1 (placebo; n=8)			Group 2 (medicated; n=8)			
Days after treatment	Ticks counted**	Mean (G)	SD	Ticks counted	Mean (G)	SD	% Control
7	244	26.83	10.3	0	0.00	0.0	100.00 *
14	279	33.73	9.5	0	0.00	0.0	100.00 *
21	244	29.98	5.7	1	0.09	0.4	99.70 *
30	217	26.76	4.8	0	0.00	0.0	100.00 *
Cum	984	29.81	8.0	1	0.02	0.2	99.93 *
* $p < 0.0001$							
** Due to a counting error in the placebo group, same number of ticks as for the evaluation at 48 hours							

efficacy of 99.67%. One day later, ie, 72 hours after re-infestation, the cumulative number of live ticks recorded was 12, resulting in a cumulative efficacy of 98.96%. Table 1 summarizes the results for each evaluation time point. No adverse drug effects were recorded and no significant cosmetic abnormalities were observed during the study. A few minor cosmetic effects (greasy hair, matted hair, white residues) present in a few dogs in both treatment groups disappeared by Study Day 3.

Study 2 (A. americanum)

After treatment, the tick recovery rates for Group 1 (placebo) ranged between 21.0% (Study Day 13) and 41.3% (Study Day 30). In the medicated group, only one live tick was recorded 48 hours after re-infestation, resulting in a cumulative efficacy of 99.79%. One day later, ie, 72 hours after re-infestation, not a single tick was recorded,. resulting in a cumulative efficacy of 100.00%. Table 2 summarizes the results for each evaluation time point. No adverse drug reactions were recorded during the study, and a only a few minor cosmetics effects appeared in a few dogs in both treatment groups disappeared after Study Day 3.

Study 3 (D. variabilis)

After treatment, the tick recovery rates for Group 1 (placebo) ranged between 54.3 (Study Day 29) and 69.8 (Study Day 13). Table 3 summarizes the results for D. variabilis. In the medicated group, a cumulative total of 19 ticks was recorded 48 hours after re-infestation, resulting in a cumulative efficacy of 98.96%. Regarding the evaluation 72 hours after re-infestation, no single living tick was recorded on Study Days 7, 14, and 30, and only one on Study Day 21, resulting in a cumulative efficacy of 99.93%. Within Group 1 (placebo), one dog vomited on Study Day 18, and two dogs had loose stools on Study Day 19 present in their cages. One animal in Group 2 (medicated) had loose stools present in its cage on Study Day 29. No apparent cosmetic effects were recorded for animals in the medicated group during the trial.

DISCUSSION

The recovery rates of at least 20% at all time-points and for all tick species indicate that the tick strains were sufficiently vigorous and that the dogs used for the trial were adequately susceptible to tick infestation.

The results of the 12.5% pyriprole spot-on solution against induced I. scapularis infestations presented here indicate a high level of efficacy (cumulatively >99% after 48 hours exposure and >98% after 72 hours exposure) maintained during the trial period of 4 weeks. This high level of sustained efficacy is comparable to the one described in a comparable investigation for two other spot-on formulations--one based on fipronil/methoprene, and the other one on permethrin/imidacloprid.[10] For an amitraz/metaflumizone spot-on, a less complete and sustained efficacy has been reported, since the percentage control assessed after 48 hours exposure of the ticks to the treated dogs on Study Days 2, 7, 12, 21, and 28 was 94.8%, 96.0%, 94.9%, 91.0%, and 82.0%, respectively[11].

In the present study, the efficacy of the 12.5% pyriprole spot-on solution against induced A. americanum infestations was similar to the one against I. scapularis, ie, a high level of protection (cumulatively > 99% after 48 hours exposure and 100% after 72 hours exposure) was maintained during the 4 weeks that followed treatment. Such a high level of sustained efficacy assessed 48 hours after re-infestation has been also reported for a permethrin/imidacloprid spot-on solution.[10] For a fipronil/methoprene spot-on however, whereas such a high efficacy was reached during the first 2 weeks after treatment, it subsequently declined down to <95% at week 3 and to <65% at week 4 after treatment.[10]

For two other permethrin spot-on solutions, the reported level of efficacy assessed between 72 and 120 hours after re-infestation was rather low 3 days after treatment (<60%); increased 1 week after treatment (>95%); but then progressively diminished down to 71.2% for one formula-

tion and to 8.3% for the other formulation 4 weeks after treatment.[12] The amitraz/metaflumizone spot-on formulation previously mentioned, did not reach such high levels of control. The efficacy during the first 3 weeks assessed after 48 hours exposure of the ticks to the treated animals was never >95% and it reached only 75.6% 4 weeks after treatment.[11]

As for I. scapularis and A. americanum, and for D. variabilis as well, the 12.5% pyriprole solution provided a very high level of control (>99% after 42 hours exposure) of the ticks to the treated animals, maintained throughout the 4 weeks that followed treatment. After 72 hours exposure, no living ticks at all were found in the medicated group on Study Days 7, 14, and 30, and only one live tick was recorded in the medicated group on Study Day 21, resulting in a cumulative efficacy of > 99%. Two comparable investigation against induced D. variabilis infestations[13,14] indicate that the already mentioned fipronil/methoprene spot-on solution shows a high initial efficacy that progressively declines until 30 days after treatment.

In one study, the percentage control assessed only on attached ticks 48 hours after re-infestation was 96.1%, 100%, 87.2%, 78.9%, and 83.2% on days 3, 7, 14, 21, and 28 after treatment, respectively.13 In the other study, percentage control also assessed only on attached ticks 48 hours after re-infestation was 99.2%, 100%, 100%, 96.1%, and 75.7% on days 3, 9, 16, 23, and 30 after treatment, respectively14 The same two studies[13,14] assessed the efficacy of the already mentioned permethrin/imidacloprid spot-on solution as well. However, the results seem inconsistent, since in one study,[13] the percentage control during the 4 weeks was rather high (100% to 92%), whereas in the other study,14 it was substantially lower (90.2% to 17.5%). The authors suggest that the low level of control in this study may have been due to tick resistance to permethrin, but they did not check the ticks for resistance.

For the amitraz/metaflumizone spot-on solution, complete control has been reported for the first 2 weeks after treatment, that clearly declined to <90% 4 weeks after treatment.[11] Finally, in an investigation comparing various treatment regimes with a selamectin spot-on solution against induced D. variabilis infestations, the efficacy of a single treatment assessed only on ticks that were attached and alive at various time-points during the first 4 weeks after treatment was rather inconsistent. In single separate studies, the percentage control achieved ranged between 64.8% and 81.9% 72 hours after re-infestation, and between 88.8% and 94.5% 120 hours after re-infestation on Study Day 9; between 0.0% and 62.3% 72 hours after re-infestation and between 30.5 and 96.5% 120 hours after re-infestation on Study Day 16; and between 0.0% and 75.4% 72 hours after re-infestation and between 16.3% and 90.0% 120 hours after re-infestation on Study Day 25. Such results suggest an initial moderate control followed by a substantial decline in the efficacy towards the end of the 4-week period.[15]

The reason for the loose stools in one dog in the medicated group on Study Day 29 during the D. variabilis Study could not be ascertained. Nevertheless, considering that it was an isolated case occurring long after treatment, and that two placebo-treated dogs also showed loose stools on Study Day 19, strongly suggest that the lose stools were not related with the medication.

In summary, the results of the three studies in the present investigation document a high level of control of the 12.5% pyriprole spot-on solution against induced I. scapularis, A. americanum and D. variabilis infestations sustained during the 30 days that followed treatment. This efficacy ranks high among other acaricidal products currently available for use on dogs. Based on these results and on the fact that the product was well tolerated by the animals, we believe that this 12.5% pyriprole spot-on solution offers an excellent tool for the control of dog infestations with ticks common in North

America.

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