

Antiparasitic Efficacy of 10% w/v Fipronil Spot-on (Fiproline Spot-on) against Experimental Tick (*Rhipicephalus sanguineus*) Infestations on Dogs

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Abstract

This study was conducted to investigate the efficacy of 10% w/v fipronil spot-on for treatment and prevention of *Rhipicephalus sanguineus* infestations on dogs. Twelve Beagles were randomly separated into two groups which were treatment and control group (6 dogs/group). The treatment group received 10% fipronil spot-on at the dosage of 6.7 mg/kg on day 0. A group of 60 *Rhipicephalus sanguineus* was released to feed on each dog on day -7, -2, 7, 14, 21, and 28. The ticks were counted and removed on days -4, 3, 10, 17, 24, and 31. Mean number of tick infestations in the control group ranged from 16.67-24.50 ticks per dog and the attachment rate ranged from 27.78-40.83%. Mean number of tick infestations in the treatment group on the days before fipronil was applied on the dogs was 21.67 ticks per dog and the attachment rate was 36.11%. Percent efficacy of fipronil used in this study was 71.77, 96.03, 100, 91.84, and 90.21% on days 3, 10, 17, 24, and 31, respectively. There were statistically significant differences between the mean numbers of tick between the control and treatment groups on days 3, 10, 17, 24, and 31. There was no significant difference of complete blood count and kidney and liver functions before and after the study. Skin rash did not show up in the treatment group after fipronil was applied on the dogs.

Keywords: brown dog tick, dog, efficacy, fipronil, spot-on

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บทคัดย่อ

ประสิทธิภาพของฟิโปรนิล 10% (ฟิโปรไลน์ สปอต-ออน) ในการกำจัดและป้องกันเห็บ

Rhipicephalus sanguineus บนสุนัข

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ศึกษาประสิทธิภาพของฟิโปรนิล 10% w/v รูปแบบหยดหลัง ในการกำจัดและป้องกันเห็บ *Rhipicephalus sanguineus* บนสุนัข การศึกษาได้ใช้สุนัขพันธุ์ปิ่นเกล้าจำนวน 12 ตัว โดยแบ่งสุนัขออกเป็น 2 กลุ่ม คือ กลุ่มทดลองและกลุ่มควบคุม (6 ตัว/กลุ่ม) ในวันที่ 0 กลุ่มทดลองจะได้รับฟิโปรนิล 10% ในขนาด 6.7 มก.ต่อกก. ปลอຍเห็บสุนัขชนิด *Rhipicephalus sanguineus* ลงบนตัวสุนัขในวันที่ -7, -2, 7, 14, 21 และ 28 และนับเห็บบนตัวสุนัขและเก็บเห็บออกจากสุนัขในวันที่ -4, 3, 10, 17, 24 และ 31 จากการศึกษาพบว่าค่าเฉลี่ยของจำนวนเห็บบนสุนัขในกลุ่มควบคุม คือ 16.67-24.50 ตัว หรือคิดเป็นร้อยละ 27.78-40.83 และค่าเฉลี่ยของจำนวนเห็บบนสุนัขในกลุ่มทดลอง ก่อนที่จะมีการหยดยา คือ 21.67 ตัว หรือคิดเป็นร้อยละ 36.11 สำหรับประสิทธิภาพของฟิโปรนิลนั้นมีค่าเท่ากับร้อยละ 71.77, 96.03, 100, 91.84 และ 90.21 ในวันที่ 3, 10, 17, 24 และ 31 ตามลำดับ และค่าเฉลี่ยของเห็บในกลุ่มทดลองและกลุ่มควบคุมในวันที่ 3, 10, 17, 24 และ 31 มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ จากการศึกษาไม่พบความผิดปกติของเม็ดเลือด ดับ และไตของสุนัขทั้งก่อนและหลัง การทดสอบ รวมทั้งไม่พบความผิดปกติของผิวหนังสุนัขภายหลังการหยดยา

คำสำคัญ: เห็บสุนัข ประสิทธิภาพ ฟิโปรนิล รูปแบบหยดหลัง

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Introduction

Rhipicephalus sanguineus or brown dog ticks are ectoparasites that can be found on dogs in both urban and rural areas worldwide including Thailand (Inokuma et al., 1995; Maroli et al., 1996; Tinoco-Gracia et al., 2009). This tick can also be found on cat and human (Uspensky and Ioffe-Uspensky, 2002; Uspensky, 2009). *Rhipicephalus sanguineus* has a three-host tick life cycle which is composed of egg, larval, nymphal, and adult stage. Larva, nymph, and adult can be found on dogs. Engorged larva and engorged nymph will leave host for molting to nymph and adult stage, respectively. Engorged female adult will also leave host for egg laying. Infestation of *Rhipicephalus sanguineus* is concerned by pet owners since the infestation can cause itch, irritation and serious blood loss. Infected ticks can also transmit pathogen during blood feeding process. *Rhipicephalus sanguineus* is an important vector for several pathogens in Thailand which are *Babesia canis*, *Ehrlichia canis*, and *Hepatozoon canis* (Baneth et al., 2001; Dantas-Torres, 2008; M'Ghirbi and Bouattour, 2008). Tick paralysis in dogs can also be caused by *Rhipicephalus sanguineus*. Therefore, the treatment and prevention of *Rhipicephalus sanguineus* infestation on dogs and other pet animals are very important.

Spot-on application is a convenient method for pet owners because they can do this by themselves at home. There are many spot-on products in veterinary market. Efficacy and price of these products are concerned by pet owners before purchasing. There are several chemicals that can be used as a spot-on application. Single or combination chemicals can be found in the present, for example, fipronil, imidacloprid, methoprene, permethrin, pyriprole, and selamectin (Denny, 2001; Doyle et al., 2005; Hagimori et al., 2005; Otranto et al., 2005; Dryden et al., 2006; Dryden et al., 2008; Schuele et al., 2008^{a,b}; Bouhsira et al., 2009). Fipronil is a phenylpyrazole antiparasitic agent which can interfere brain and spinal cord of the ticks, resulting in death of the ticks. Fipronil acts by binding to gamma-aminobutyric acid (GABA) and glutamate receptors. It inhibits the opening of the chloride ion channels and leads to neuronal hyperactivity (Hosie et al., 1995; Hainzl and Casida, 1996; Narahashi et al., 2010). Fipronil has efficacy against various species of fleas, lice, and ticks, for example, *Ctenocephalides canis*, *Ctenocephalides felis*, *Trichodectes canis*, *Dermacentor reticulatus*, *Rhipicephalus sanguineus*, and *Ixodes ricinus* (Cooper and Penaliggon, 1996; Ritzhaupt et al., 2000; Pollmeier et al., 2002; Oliveira et al., 2009). This study was conducted to investigate the efficacy of 10% w/v fipronil spot-on for treatment and

prevention of brown dog tick (*Rhipicephalus sanguineus*) infestations on dogs in Thailand.

Materials and Methods

Experimental animals: Twelve Beagles of both sexes and with various weights were used in this study. They were randomly separated into two groups which were treatment and control group (6 dogs/group). This study was approved by the Chulalongkorn University Animal Care and Use Committee (Animal Use Protocol and Approval No. 11310068).

Ticks: Thailand strain of *Rhipicephalus sanguineus* was used in this study. They were reared and maintained at the parasitology laboratory, Parasitology Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University. Two- to three-week-old adult ticks were used in this study.

Tested substance: 10% w/v fipronil spot-on (Fiproline Spot On, Thainaoka Pharmaceutical, Thailand, Lot. No. R&D 17/09/11 Mfg. Date 26/09/2011) was used in this study. A dose of 6.7 mg fipronil per kg bw was applied on the skin of the dogs at the base of the neck between the shoulder blades once only on day 0 in the treatment group.

Experimental design: There were 12 dogs in this study and they were randomly separated into two groups (6 dogs/group). All dogs were free from any acaricide for at least two months. The dogs were bathed with non-acaricide shampoo about two weeks before the study was started.

The control group did not received any fipronil during the study but the treatment group received Fiproline Spot On on day 0. A group of 30 males and 30 females of *Rhipicephalus sanguineus* was allowed to feed on each dog on days-7, -2, 7, 14, 21, and 28. The ticks were counted and removed on days-

4, 3, 10, 17, 24, and 31. Mean numbers of ticks between the control and treatment groups were compared by using t-test and fipronil efficacy was calculated as the following formula.

$$\text{Fipronil efficacy (\%)} = ((\text{Geometric mean control} - \text{Geometric mean treatment}) / \text{Geometric mean control}) \times 100$$

Geometric mean control = geometric mean number of ticks on untreated control dogs at each individual assessment day

Geometric mean treatment = geometric mean number of ticks on fipronil treated dogs at each individual assessment day

Blood was collected from each dog before and after the study. It was tested for complete blood count, creatinine, BUN, SGPT, and alkaline phosphatase to indicate kidney and liver functions. Skin rash on the treatment group was also investigated. Tick infestations were taken to be successful when the attachment rates (attached/applied) were equal or more than 25%. For all analyses, a *p*-value threshold was set to 0.05.

Results and Discussion

There were 12 Beagle in this study and they were randomly separated into two groups (6 dogs/group). The control group did not receive any fipronil during the study but the treatment group received fipronil spot-on. Body weight of the control group ranged from 9.9-14.9 kg and body weight of the treatment group ranged from 7.3-12.4 kg.

A group of 30 males and 30 females of *Rhipicephalus sanguineus* was released to feed on each dog on days-7, -2, 7, 14, 21, and 28. The ticks were counted and removed on days-4, 3, 10, 17, 24, and 31. The results are shown on Table 1 and 2.

Table 1 Number of *Rhipicephalus sanguineus* found on the control group on days-4, 3, 10, 17, 24, and 31

Tick released day	Tick counted day	Control group					
		Dog#1	Dog#2	Dog#3	Dog#4	Dog#5	Dog#6
Day-7	Day-4	7	18	15	22	19	19
Day-2	Day 3	29	19	12	16	23	25
Day 7	Day 10	19	18	16	11	28	34
Day 14	Day 17	27	10	16	8	30	28
Day 21	Day 24	27	25	21	10	36	28
Day 28	Day 31	30	18	17	20	30	28

Table 2 Number of *Rhipicephalus sanguineus* found on the treatment group on days-4, 3, 10, 17, 24, and 31, which received Fiproline Spot On at the dose of 0.67 ml/10 kg body weight (equivalence to fipronil 6.7 mg/kg) on day 0

Tick released day	Tick counted day	Treatment group					
		Dog#7	Dog#8	Dog#9	Dog#10	Dog#11	Dog#12
Day-7	Day-4	12	24	21	21	25	27
Day-2	Day 3	0	7	9	6	12	1
Day 7	Day 10	0	0	0	3	2	0
Day 14	Day 17	0	0	0	0	0	0
Day 21	Day 24	2	0	4	6	0	0
Day 28	Day 31	1	0	3	4	2	4

Mean number of tick infestations in the control group ranged from 16.67-24.50 ticks per dog and the attachment rate ranged from 27.78-40.83% (mean of 35.14%). Mean number of tick infestations in the treatment group on the days before fipronil was applied on the dogs was 21.67 ticks per dog and the attachment rate was 36.11%. Mean numbers of ticks between the control and treatment groups were compared on day-4 to indicate that there was no difference between tick feeding or attachment between the groups of the dogs. In this study, there was no statistically significant difference of mean number of ticks between the control and treatment groups on day-4 ($p = 0.1307$).

Percent efficacy of Fiprolone Spot-on used in this study was 71.77, 96.03, 100, 91.84, and 90.21% on days 3, 10, 17, 24, and 31, respectively (Table 3). There were statistically significant differences between the mean numbers of tick between the control and treatment groups on days 3, 10, 17, 24, and 31 ($p < 0.05$). There was no significant difference of complete blood count and kidney and liver functions before and after the study. Skin rash did not show up in the treatment group after fipronil was applied on the dogs.

Not all released ticks would attach and feed on the dogs. In this study, we found that the mean number of tick infestations, indicating vigorous tick challenges on all assessment days (EMEA, 2007) in the control group ranged from 16.67-24.50 ticks per dog and the attachment rate ranged from 27.78-40.83%. Other studies showed that *Dermacentor reticulatus* attachment rate on dogs were between 29.7-59.6% and *Ixodes ricinus* attachment rate on dogs were between 37.0-50.7% (Bonneau et al., 2010; 2011).

The efficacy of this product against the attached ticks on dogs was 71.77% on day 3 after the product was applied on the dogs. The efficacy of this product on days 10, 17, 24, and 31 after the product was applied on the dogs were higher than 90% with the range between 90.21-100%. Accordingly, if ticks of *Rhipicephalus sanguineus* are present when the product is applied, all the ticks may not be killed within the first 72 hours but they may be killed within a week. The highest efficacy of this product was on day 17, after the product was applied on the dogs, and it decreased to 90.21% on day 31. The study indicated that this product had the persistent efficacy against

Rhipicephalus sanguineus infestations on the dogs for at least 4 weeks.

The study by Guerrero (2010) indicated that the arithmetic mean of *Rhipicephalus sanguineus* attachment for the untreated control group of dogs ranged from 18.8/50 to 34.3/50. This study also showed that the efficacy of PetArmor® (9.7% w/w fipronil) against *Rhipicephalus sanguineus* was 51.3, 98.4, 99.6, 99.6, and 97.5% on days 2, 9, 16, 23, and 32, respectively, and the efficacy of Frontline® Top Spot (9.7% w/w fipronil) against *Rhipicephalus sanguineus* was 65.0, 100.0, 98.0, 94.9, and 76.1% on days 2, 9, 16, 23, and 32, respectively.

The efficacy of fipronil reported in this study is similar to the efficacy of other formulations of the fipronil. Bonneau et al. (2010) found that the efficacy of one fipronil formulation (Frontline®) against *Ixodes ricinus* was 98.8, 100, 100, 100, 86.3, and 77.2% on day 2, 9, 16, 23, 30, and 37 after treatment, respectively, and the efficacy of another fipronil formulation (Effipro®) against *Ixodes ricinus* was 93.8, 100, 100, 98.9, 97.9, and 94.1% on day 2, 9, 16, 23, 30, and 37 after treatment, respectively.

Bonneau et al. (2011) also found that the efficacy of one fipronil formulation (Frontline®) against *Dermacentor reticulatus* was 99.2, 100, 99.1, 96.2, 97.3, and 89.8% on days 2, 42, 44, 51, 58, and 65 after treatment, respectively, and the efficacy of another fipronil formulation (Effipro®) against *Dermacentor reticulatus* was 98.3, 100, 97.4, 98.8, 96.3, and 85.6% on days 2, 42, 44, 51, 58, and 65 after treatment, respectively.

Fipronil has been used as insecticide and acaricide in the veterinary market as a spray and spot-on formulation for more than ten years. Some veterinarians and pet owners might worry about the efficacy of fipronil against tick infestations on dogs. This study not only indicated the efficacy of this fipronil product but also indicated that fipronil still had the efficacy against *Rhipicephalus sanguineus* infestations on dogs in Thailand. Although the addition of S-methoprene prevents flea eggs from hatching and larvae from developing into egg-laying adults, formulation containing only fipronil continues to provide excellent efficacy against fleas and ticks (Guerrero, 2010). The efficacy of fipronil against fleas and lice on dogs in Thailand still needs to be investigated.

Table 3 Percent efficacy of 10% w/v fipronil spot-on (Fiprolone Spot On, Thainaoka) against *Rhipicephalus sanguineus* on dogs on days 3, 10, 17, 24, and 31

Tick released day	Tick counted day	Mean number of ticks		Tick attachment rate		Percent Efficacy	p-value*
		Control group	Treatment group	Control group	Treatment group		
Day-7	Day-4	16.67	21.67	27.78	36.11		0.1307
Day-2	Day 3	20.67	5.83	34.45	-	71.77	0.0009
Day 7	Day 10	21.00	0.83	35.00	-	96.03	0.0002
Day 14	Day 17	19.83	0.00	33.05	-	100.00	0.0005
Day 21	Day 24	24.50	2.00	40.83	-	91.84	0.0001
Day 28	Day 31	23.83	2.33	39.72	-	90.21	<0.0001

*Comparison of mean numbers of ticks between control and treatment groups

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