



[原著]

Tickicidal efficacy of a spot-on formulation of fipronil directly applied against tick dorsal side

Usability as a supplementary method for treating tick infestation in dogs

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Summary

Ticks occur frequently on dogs, to which they often transmit pathogens of many types, including zoonotic pathogens. Therefore, they should be eliminated quickly. This study evaluated direct application of a spot-on formulation of fipronil to ticks' dorsal side. For this study, 128 ticks on 30 dogs were treated with the formulated fipronil at a dosage of 5 μ L or 500 μ g active ingredient. For 104 ticks on the other 30 dogs, a proper dosage was applied with a cotton swab. Within 3 hr, 94% and 96% ticks were eliminated, respectively: all were eliminated within 6 hr. No adverse event was observed for any dog. Direct application of this drug was adopted as a complementary treatment of tick infestation.

Keywords: dog, fipronil, tick

Introduction

Ticks are important ectoparasites encountered in veterinary practice and in human medicine. They not only cause damage directly to the skin of host animals; they transmit many pathogens, including zoonotic pathogens (Madison-Antenucci et al., 2020; Rochlin and Toledo, 2020; Cutler et al., 2021). Therefore, elimination of ticks from animals is an urgent task not only for veterinary practice, but also for

prevention of zoonoses from the viewpoint of public health.

Tickicides of many kinds have been developed for dogs and domestic cats. Spot-on administration, usually applied to the skin between the left scapula and right scapula on the dorsal side of the neck of a dog or cat, is one tickicide administration route. Spot-on formulations of two types are used: one type develops its efficacy by absorption of the active ingredient into the blood of

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animals after medication, as those using selamectin (Bishop et al., 2000); the other type is generally regarded as developing its efficacy through distribution of the active ingredient to the animal body surface from the spotted area, as those using fipronil (Cruthers et al., 2001) and pyriprole (Schuele et al., 2008). The drug of the former type acts as oral poison against ectoparasites. The latter type acts as a contact poison.

With the latter type of spot-on formulation, active ingredients spread gradually on the animals' body surface. Their density is probably lower at areas farther from the drug-applied area. Accordingly, the possibility exists that sufficient amounts of the active ingredient to develop the tickicidal effect will not be distributed at distal areas such as the tip of fore-legs and hind-legs. The report described earlier that a pour-on formulation using flumethrin as an active ingredient developed excellent tickicidal efficacy when applied directly to the tick dorsal side (Nakamura and Fukase, 2021). However, this product was intended originally for cattle. It is not always readily available at veterinary hospitals providing care chiefly for dogs and cats. Therefore, for the present study, we examined the efficacy of another tickicidal drug, a spot-on formulation of fipronil that had been used widely in small animal practice, when applied directly to ticks.

Materials and Methods

Drug

The evaluated drug is a spot-on formulation containing fipronil, a phenylpyrazole compound, as an active ingredient, "Frontline Spot On Dog" (Boehringer Ingelheim Animal Health Japan Co., Ltd., Tokyo, Japan). The drug, which contains 10% (W/ V)

fipronil or 10 g of fipronil in 100 mL of the product, has been used widely as an ectoparasiticide against fleas and ticks on dogs (Cruthers et al., 2001).

Animals

For this study, 60 dogs of various breeds, 28 females (21 had been ovariectomized or ovariohysterectomized) and 32 males (26 had been orchietomized), 5 months to 13 years old, weighing 3–32 kg, which were kept privately and which were naturally infested by four or more ticks in various areas of Japan; Saitama, Tokyo, Kanagawa, Osaka, and Hyogo prefectures, in addition to Okinawa prefecture (Ryukyu Islands). No dog had been administered any ectoparasiticide, at least during the prior one year.

Procedure of experiment 1

For this study, two experiments were conducted. In experiment 1, for which the exactly measured volume of the drug was administered, participating animals were 30 of the 60 dogs, 15 females (11 had been ovariectomized or ovariohysterectomized) and 15 males (12 had been orchietomized), 6 months to 12 years old, weighing 3–32 kg. Two ticks on each dog, i.e., 60 ticks on the 30 dogs, were treated as unexposed control subjects, whereas the other 128 ticks were exposed to the drug. These ticks were all adults and did not reach engorgement. The drug was applied directly to the dorsal side of these exposed ticks at a dose of 5 μ L of the product or 500 μ g of the active ingredient per tick using a micropipette. The ticks were then observed at 3, 6, 12 and 24 hr after exposure.

Procedure of experiment 2

Subsequently, we conducted experiment 2, for which the unmeasured proper volume of the drug was administered, and examined the other 30

Table 1. Efficacy of a directly applied spot-on formulation containing fipronil as an active ingredient against ticks on dogs when applied directly to their dorsal sides using a micropipette with a dose of 5 μ L

Treatment	No. of ticks treated	No. (%) of ticks eliminated after			
		3 hr	6 hr	12 hr	24 hr
Unexposed	60	0	0	0	0
Exposed	128	120 (94%)	128 (100%)	128 (100%)	128 (100%)

dogs, 13 females (10 had been ovariectomized or ovariorectomized) and 17 males (14 had been orchietomized), 5 months to 13 years old, weighing 4–28 kg. The experiment design was the same as that used for experiment 1, except for application of the drug. The drug was applied directly to the dorsal side of ticks of the exposed group at a proper dosage using a cotton swab that had been immersed in the drug solution, assuming an actually applicable method in a clinical scenario. The treated ticks were all adults and did not engender engorgement. The ticks were then observed at 3, 6, 12 and 24 hr after exposure.

Confirmation of the killing effect of the drug

The eliminated ticks in both experiments were collected to the greatest extent possible and were observed to move or not. They were then put into Petri dishes on the bottom of which a piece of filter paper was seated for 24 hr to be confirmed not to revive. At this time, ticks that did not move at all, even when pricked with a needle, were judged as dead.

Observation of the tick morphology

The collected ticks were observed to assess their morphology, confirming that

they had their gnathosomas, or had left these organs on the dog body. Moreover, the tick species were identified based on their morphology.

Observation of adverse events

The dogs were observed carefully, especially their skin and hair coat around sites that ticks had infested, in addition to general findings obtained during three days after medication.

Ethics

The dogs had all entered the study after agreement of their guardians, and were treated with consideration of animal welfare during the study, based on the Regulation on Animal Experimentation at the General Incorporated Association Katsuragi Institute of Life Sciences.

The ticks remaining on the dogs' body surface at the termination of this study were eliminated completely by administering a spray formulation of fipronil, "Frontline Spray" (Boehringer Ingelheim Animal Health Japan Co., Ltd., Tokyo, Japan).

Results

From experiment 1, 188 ticks were detected on the 30 dogs, on which the number of ticks detected from a dog were 4–10 with a median of 6. Of the 188 ticks, 128 ticks were exposed to the drug

Table 2. Efficacy of a directly applied spot-on formulation containing fipronil as an active ingredient against ticks on dogs when applied directly to their dorsal sides using a cotton swab with proper dosage

Treatment	No. of ticks treated	No. (%) of ticks eliminated after			
		3 hr	6 hr	12 hr	24 hr
Unexposed	60	0	0	0	0
Exposed	104	100 (96%)	104 (100%)	104 (100%)	104 (100%)

at a dosage of 5 μ L (500 μ g as the active ingredient) of the fipronil remedy. Results of experiment 1 show that, of the exposed 128 ticks, 120 (94%) ticks were eliminated (fell or knocked down) within 3 hr. The remaining 8 ticks were eliminated within 6 hr. The unexposed 60 ticks, by contrast, were observed to infest the dogs during the 24 hr observation period (Table 1).

From experiment 2, 164 ticks were detected on the 30 dogs. The numbers of ticks detected from a dog were 4–9, with a median of 5.5. Accordingly, 104 of the 164 ticks were exposed to the drug at a proper dosage. Results indicate that 100 (96%) of the 104 ticks were eliminated (fell or knocked down) within 3 hr; the remaining four ticks were eliminated within 6 hr. The unexposed 60 ticks, by contrast, were observed to infest the dogs during the 24 hr observation period (Table 2).

The eliminated and collected ticks were observed not to move. At 24 hr after being put into Petri dishes, all ticks were confirmed as not having revived: the ticks were all dead.

Morphological observation revealed that all the eliminated ticks had their gnathosomas, and had not left these organs on the dog body. Most ticks were identified as *Haemaphysalis longicornis* and *H. flava* in areas except for Okinawa

prefecture (Ryukyu Islands), where most were *Rhipicephalus sanguineus* (Table 3).

No adverse event was detected in the 60 medicated dogs from experiments 1 or 2.

Discussion

This study demonstrated that direct application of the fipronil product to tick dorsal sides can eliminate them within a short period. As far as observed in this study, the efficacy was regarded as killing, based on the fact that the eliminated ticks did not revive. Efficacies of the drug were also thought not to be different among tick species because the exposed ticks were all eliminated. In addition, the 8 and 4 ticks observed respectively in experiments 1 and 2 that took more time for disinfestation than the other tick individuals were all *H. longicornis*; almost all of *H. longicornis* had been eliminated within a shorter time.

In this study, many of the examined ticks were species having a shorter mouthpart. When the drugs are applied directly to the dorsal side of tick species, such as *Ixodes persulcatus*, having a longer mouthpart, the ticks might not fall so easily as in this study. However, the drug will kill even ticks of such species.

Table 3. Species of ticks collected after elimination*1

Tick Species	Experiment 1		Experiment 2	
	No. of ticks collected	Prevalence (%) ^{*2}	No. of ticks collected	Prevalence (%) ^{*2}
<i>Haemaphysalis longicornis</i>	29	40	26	39
<i>Haemaphysalis flava</i>	21	29	20	30
<i>Haemaphysalis campanulata</i>	3	4	2	3
<i>Ixodes ovatus</i>	2	3	1	1
<i>Rhipicephalus sanguineus</i> ^{*3}	17	24	18	27

*1 Ticks were collected to the greatest extent possible: 72 and 67 ticks were obtained respectively from experiment 1 and 2.

*2 Prevalence (%) = [No. of ticks collected/ Total No. of ticks collected (72 or 67)] × 100

*3 Obtained only from Okinawa prefecture (Ryukyu Islands)

When comparing this result to that obtained our earlier study with flumethrin (Nakamura and Fukase, 2021), the efficacy of the directly applied fipronil product was almost equivalent to that of the flumethrin remedy. Therefore, direct application of fipronil spot-on formulation, as well as flumethrin pour-on formulation, to the tick dorsal side can be adopted as a complementation, although with the proviso that the method is applicable only against ticks with a body size to which the drug is directly applicable.

Conflict of Interest

The authors declare that they have no conflict of interest associated with this article or the study it describes.

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犬に寄生するマダニの背面へ直接塗布したフィプロニルのスポットオン式滴下投与用液剤の殺ダニ効果 —犬に寄生するマダニの駆除に際しての補助的方法としての有用性—

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要旨

マダニは人獣共通感染症の病原体を媒介することが多く、犬に寄生するマダニの駆除は迅速に行う必要がある。しかし、犬のマダニ駆除薬として用いられているスポットオン式滴下投与用液剤の有効成分は、犬の体表に均等に分布するとは限らず、様々な部位における分布濃度に濃淡が生じると考えられる。本研究は、マダニ体表への殺ダニ剤の直接の塗布がその駆除のための補助的な方法として有用であることを確認するために実施した。マダニの自然感染を受けている犬 60 頭を供し、このうち 30 頭に寄生していたマダニ計 128 個体に対してはフィプロニルのスポットオン式滴下投与用液剤を 1 個体あたり製剤として 5 μ L (有効成分として 500 μ g) の用量で、他の 30 頭に寄生していた 104 個体に対しては同薬剤の適量を綿棒に浸潤させて、それぞれ背面に直接的に塗布した。その結果、各々の方法で 94% と 96% のマダニが 3 時間以内に、他のマダニも 6 時間以内に駆除された。また、有害事象はいずれの犬にも観察されなかった。以上のことから、マダニ背面へのフィプロニルの滴下投与用液剤の直接の塗布は、マダニの駆除に際しての補助的な方法であると結論した。

キーワード: 犬、フィプロニル、マダニ