

Inhibition and Prevention Efficacy against Mosquito Bloodsucking and *Dirofilaria immitis* Infection by Administration of Topical Insecticide

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ABSTRACT. Inhibition and prevention of mosquito bloodsucking by a topical insecticide, and the consequent infection of *Dirofilaria immitis* were evaluated using a product containing 10% w/w imidacloprid plus 50% w/w permethrin (I-P solution). Nine healthy beagle dogs divided in three groups of 3 dogs each were administered I-P solution on day 3 (group 1), day 36 (group 2) and not administered (group 3 as a control) before starting the experimental infection. The results showed that the mosquito bloodsucking rate was significantly lower, 12.1% in group 1 ($p < 0.05$) and 11.1% in group 2 ($p < 0.05$), than the 40.6% in group 3, meaning that the inhibition rates in groups 1 and 2 against group 3 were 70.2% and 72.7%. The infection rate of experimental *D. immitis* infection was 33% in group 1, 33% in group 2 and 100% in group 3, meaning that the prevention rates in groups 1 and 2 against group 3 were 67% and 67%.

KEY WORDS: canine, *Dirofilaria immitis* infection, mosquito bloodsucking, topical insecticide.

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A spot-type insecticide solution containing both imidacloprid and permethrin (I-P solution) is expected to show, not only an insecticidal but also a bloodsucking repellent efficacies against mosquito biting, consequently, preventing *Dirofilaria immitis* infection. Only a few reports, however, have studied on the bloodsucking inhibitory efficacy of imidacloprid and permethrin against ticks and fleas [1, 4-6, 8], and mosquitoes [1, 7].

This study was conducted to assess the inhibitory efficacy of I-P solution against bloodsucking by mosquitoes and the preventive efficacy to *D. immitis* infection by an experimental infection procedure.

MATERIALS AND METHODS

Dogs and experimental groups: Nine healthy female beagles were obtained commercially (Nihon Nousan Kogyo Co., Ltd., Tokyo)(weight between 7 and 11 kg, age 9 months) and confirmed to be free of *D. immitis* infection by the Knott test for microfilaria and a commercial antigen-detecting ELISA kit for adult worms. They were also examined by physical, hematological, blood chemistry and fecal examinations, and confirmed as normal. They were then vaccinated with a commercial mixed vaccine for dog use, housed together in a single kennel with open windows, and fed commercial dog food with free access to clean water throughout the experimental period from June 2006 to January 2007, at the laboratory animal facility (School of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan). Groups 1 and 2 were administered I-P solution on day 3 and day 36 before the experimental *D. immitis* infection, respec-

tively, and group 3 served as a control of the experimental infection without administration of I-P solution.

I-P solution: The solution contains a combination of 10% imidacloprid (100 mg/ml) and 50% permethrin (500 mg/ml) (Fortreon™, Bayer Yakuin Co., Ltd., Tokyo) and the standard dose is to drop 0.1 ml/kg of the solution on the interscapular portion of the back. According to the package insertion, the solution penetrates the sebum and spreads over the skin surface throughout the body within 2 days, without absorption into skin, and the insecticidal efficacy lasts about one month against flea and ticks.

Bloodsucking rate and blood weight sucked by mosquitoes: Numerous mosquitoes, *Aedes togoi*, hatched from larvae, were placed in 9 small cages covered with steel mesh net, and then male mosquitoes were removed by aspiration. The remaining female mosquitoes were kept in a dark and quiet room to avoid injury until use. The number of female mosquitoes in each cage was about 100 to 150. The cage containing the mosquitoes was placed on the chest of a dog, after clipping the hair and under general anesthesia, and the mosquitoes sucked blood for about 30 min through the steel mesh net. Each mosquito in the cage was then weighed on an electronic balance after freezing in a freezer, and blood-sucking was confirmed by crushing each mosquito on moist filter paper. This procedure was performed individually using the nine beagle dogs in groups 1, 2 and 3 within one day. The I-P solution had been administered to group 1 on day 3 and to group 2 on day 36 prior to this procedure.

The bloodsucking rate (mosquitoes that sucked blood / whole mosquitoes used \times 100) per cage and the mean body weight of mosquitoes that sucked blood per cage were calculated to obtain an average value in each group. The mean blood weight sucked was measured by subtracting the mean body weight value of non-sucked female mosquitoes, independently preserved as a control, from the mean body

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weight value of mosquitoes that sucked blood.

Experimental *D. immitis* infection: The experimental infection of nine dogs was performed using a previous procedure [2, 3] within one day. The microfilaremic dog, an approximately one-year-old mixed breed female, used in this experiment was born and lived in Yamaguchi prefecture and was naturally infected with *D. immitis*. About 100 to 150 newly hatched female mosquitoes bit the microfilaremic dog, and were collected in several cages. Ten days later, after confirming that infective larvae (L3) of *D. immitis* were located in their proboscis portion by crushing a few mosquitoes in saline, the cages were used for infection. The cage was placed on the chest of a dog, after clipping the hair and under general anesthesia, and the experimental infection was performed by allowing the mosquitoes to again suck blood for about 30 min through the steel mesh net, although it was not possible to confirm the number of L₃ which invaded the beagle dogs. The infection was performed individually for nine beagle dogs in groups 1, 2 and 3.

Serial administration of the solution and recovery of infected worms: According to the experimental schedule (Fig. 1), groups 1 and 2 were administered I-P solution once every 30 days after the experimental infection on day 0 until the end of the experiment. The dose was often adjusted individually according to the increased body weight of the beagle dogs. The windows of the kennel remained open to increase the chance of spontaneous *D. immitis* infection by wild mosquitoes naturally infected with *D. immitis* L₃ invading the kennel. On day 141, nine beagle dogs under-

went euthanasia with sodium pentobarbital, and then the right ventricle and pulmonary arteries were immediately dissected and examined for the worm. The thoracic and abdominal cavities were also examined for an ectopic infection of the worm.

Statistic analysis: The bloodsucking rate among the groups was analyzed using Student's *t* test.

RESULTS

Inhibitory efficacy on bloodsucking rate and blood weight sucked by mosquito: As shown in Table 1, the mean bloodsucking rate was significantly decreased in group 1 ($p < 0.05$) and 2 ($p < 0.05$) as compared with group 3, with the inhibitory efficacy [group 3 (40.6%) - group 1, 2 (12.1%, 11.1%)/group 3 (40.6%) \times 100] of I-P solution to bloodsucking by mosquitoes being 70.2% in group 1 and 72.7% in group 2.

The mean blood weights sucked by one mosquito, measured by subtracting 24 non-sucking female mosquitoes as a control group, are shown in Table 1. There were no significant differences among the three groups.

Prevention efficacy on *D. immitis* infection by mosquito: As shown in Table 2, all nine experimental dogs were examined on day 141 to detect *D. immitis* worms after euthanasia. Three worms (2 males and 1 female) were detected from the right ventricle and pulmonary arteries in dog no. 2 in group 1, 1 worm (1 male) in dog no. 6 in group 2, while all 3 dogs in group 3 were infected; 1 worm (1 male) in dog no. 7, 1

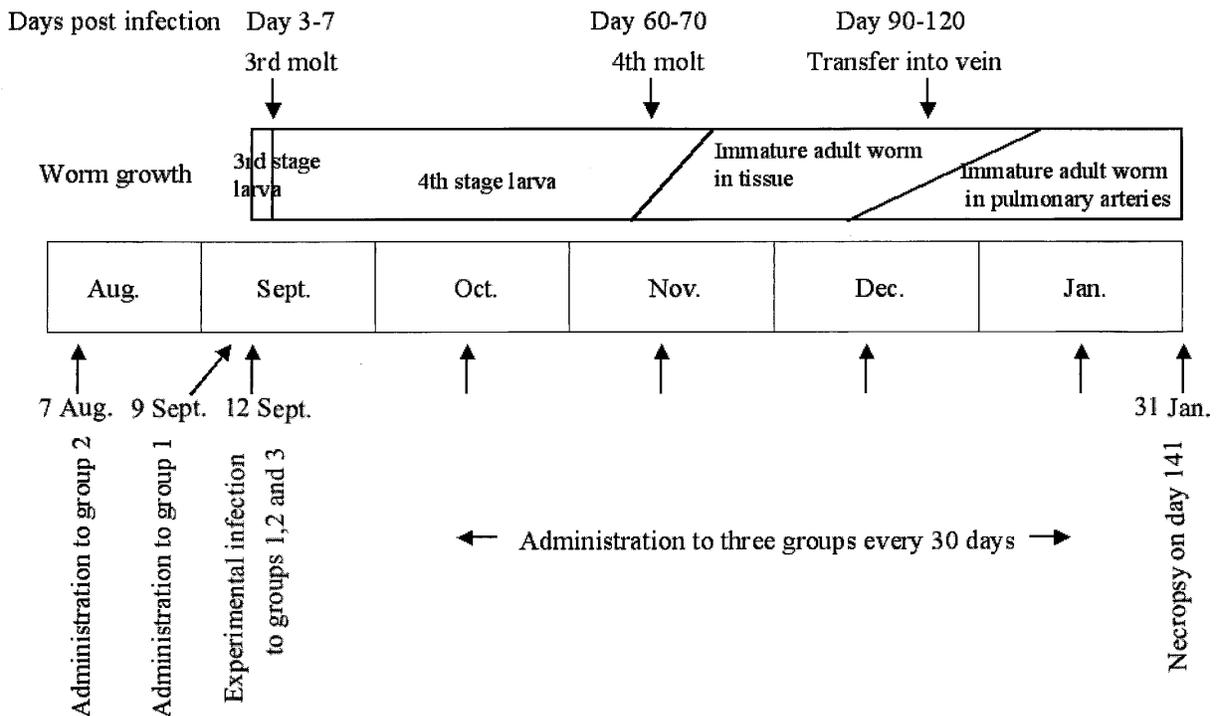


Fig. 1. Schematic schedule of the experimental infection and the administration of I-P solution.

Table 1. Inhibitory efficacy of I-P solution against bloodsucking by mosquitoes

Group	Control	1	2	3
Mean bloodsucking rate ^{a)}		12.1%	11.1%	40.6%
Mean weight per mosquito that sucked blood		3.71 mg	3.09 mg	3.53 mg
Mean weight per mosquito that did not suck blood	2.81 mg			
Mean blood weight per mosquito that sucked blood ^{b)}		0.9 mg	0.28 mg	0.72 mg

a) Number of mosquitoes that sucked blood /number of mosquitoes tested.

b) Mean weight per mosquito that sucked blood—mean weight per mosquito that did not suck blood.

Table 2. Preventive efficacy of I-P solution against *Dirofilaria immitis* infection

Dog	No. of worms detected (M/F)	Worm length (cm)	No. of dogs infected /No. of dogs tested (Infection rate)	Inhibition rate
Group 1			1/3 (33%)	67%
No. 1	0			
No. 2	3 (2/1)	M: 13.5, 15.0, F: 20.5		
No. 3	0			
Group 2			1/3 (33%)	67%
No. 4	0			
No. 5	0			
No. 6	1 (1/0)	M: 14.0		
Group 3			3/3 (100%)	0%
No. 7	1 (1/0)	M: 10.0		
No. 8	1 (0/1)	F: 17.0		
No. 9	4 (3/1)	M: 11.0, 13.0, 13.5, F: 16.0		

M: Male, F: Female.

worm (1 female) in dog no. 8 and 4 worms (3 males and 1 female) in dog no. 9. From the results, the infection rate (no. of dogs infected/no. of dogs tested \times 100) in each group was 33%, 33% and 100%, respectively, indicating that the preventive efficacy was 67%, 67% in groups 1 and 2, while 0% in group 3. The worms detected were identified as adult worms by measuring their body length. Ectopic infection with *D. immitis* was not detected in the body cavities.

DISCUSSION

The experimental dogs were kept in a kennel with open windows in anticipation of spontaneous bites by wild mosquitoes potentially infected with *D. immitis*, and the potential reduction of efficacy of the I-P solution by means of frequent contact, huddling and licking among them. The results revealed that the I-P solution effectively inhibited bloodsucking by mosquitoes.

In contrast, the mean blood weights sucked by a mosquito in groups 1, 2 and 3 were varied, although statistical analysis indicated no significant differences among groups. The result may indicate that the inhibitory efficacy of the I-P solution is not necessarily stable in the first several days after administration, because of the potential variation in time to spread over the skin surface of the body among dogs.

Another report [1] has described that the mean bloodsucking rate by mosquitoes was 1.9% on day 1, 9.7% on day 21 and 20.2% on day 28 after administration of the I-P solution, respectively. The former two values were lower than our rate (12.1%) on day 3 in group 1; however, on day 28 it

was higher than our rate (11.1%) on day 36 in group 2. These results indicate that the efficacy of I-P solution lasts for at least one month after administration.

No study has examined an efficacy of the I-P solution in relation to the inhibition of mosquito bloodsucking and the consequent prevention of *D. immitis* infection. The present study revealed that the I-P solution was moderately effective in the prevention of *D. immitis* infection by inhibiting mosquito biting. The mean prevention rate of *D. immitis* infection was 67% (Table 2). The prevention of *D. immitis* infection may involve the combination of a repellent effect and an insecticide effect. Additionally, a larvicide effect against L₃ may be an interesting area for further study of the I-P solution because L₃ leave the proboscis of the infected mosquitoes and come in contact with the dog's skin, when an infected mosquito bites a dog.

Interestingly, the bloodsucking rate was similar between groups 1 (11.1%) and 2 (12.1%), despite the 36-day interval between administrations. Thus the efficacy may last more than one month without any substantial reduction.

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