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ORIGINAL PAPER

Repellent and adulticide efficacy of a combination containing 10% imidacloprid and 50% permethrin against *Aedes aegypti* mosquitoes on dogs

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Abstract This study was conducted to assess the repellent and adulticide efficacy of the combination containing 10% imidacloprid and 50% permethrin against *Aedes aegypti* mosquitoes on dogs. Blood-feeding success rates of the mosquitoes that were exposed to the treated dogs were 4.9 and 4.4% on days 3 and 7 post the combination application (PCA), respectively, and blood-feeding success rates increased to 6.3, 12.8, and 24.5% on days 14, 21, and 28 PCA, respectively. Blood-feeding success rates between the mosquitoes that were exposed to the treated and untreated control dogs on days 3, 7, 14, and 21 PCA were significantly different. All mosquitoes that were exposed to the treated dogs on day 3 PCA died, and mortality rates decreased to 97.1, 77.8, 40.4, and 2.1% on days 7, 14, 21, and 28 PCA, respectively. Mortality rates between the mosquitoes that were exposed to the treated and untreated control dogs on days 3, 7, 14, and 21 PCA were significantly different. This study suggested that this combination can be used to repel and kill mosquitoes on dogs; however, the application of this insecticide combination on dogs needs to be repeated every 3–4 weeks.

Introduction

Imidacloprid is a chloronicotinyl nitroguanidine synthesized from the nitromethylene class of compounds since 1985. Its chemical name is 1-{(6-chloro-3-pyridinyl) methyl}-*N*-nitro-2-imidazolidinimine. It has an activity against a wide variety of insects, novel mode of action, excellent residual activity, and low mammalian toxicity. Imidacloprid was registered for use in the UK and USA in 1993 and 1994, respectively. Permethrin is a synthetic pyrethroid that was first synthesized in 1973. The chemical name of permethrin is 3-phenoxybenzyl (1RS)-cis, trans-3-(2,2 dichlorvinyl)-2,2 dimethlycyclopropanecarboxylate. It has a broad spectrum against insects and arachnids, and it was used against a wide variety of pests and for agricultural purposes.

The combination of 10% imidacloprid and 50% permethrin was recently registered in the USA and other countries, including Thailand markets. This combination is indicated for the prevention and treatment of ticks, fleas, and mosquitoes on dogs.

Aedes aegypti (L.) is one of the most widespread floodwater mosquitoes. They are recognized as the most serious mosquitoes due to their abundance, widespread distribution, and breeding potential in floodwater habitats. They readily feed on humans and animals, so they might play an important role as bridge vectors for many pathogens between humans and animals. The most serious mosquito-borne disease in dogs is dirofilariosis that is caused by *Dirofilaria immitis* (Leidy 1856). Epidemiological studies on this disease in Thailand suggest that there is a high incidence of *D. immitis* in both stray and domestic dogs (Sangkavoranond 1981; Niwetpathomwat et al. 2006). Mosquitoes are the only natural vector for canine dirofilariosis, with approximately 70 mosquito species being implicated. All dogs living in a highly populated mosquito area are at risk. Dirofilariosis is not the only life-threatening disease that occurs in dogs, but this filarial nematode can also cause pulmonary nodules, pulmonary granulomas, or subcutaneous nodules in humans (Rena et al. 2002; Tsung

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and Liu 2003; Mumtaz et al. 2004). Recently, the attempt to control canine dirofilariosis in Thailand is the monthly injection with ivermectin to eliminate infective larval stages of *D. immitis* before patency. Without the prevention of mosquito biting, mosquitoes will be very annoying for dogs, and some degree of blood loss will happen in dogs, particularly in puppies. Insecticide that can prevent mosquito biting will be very helpful for the pet owners to prevent mosquito-borne pathogens in their pets. Assessment of insecticide efficacy (IE) conducted in an endemic area needs to be addressed.

This study was conducted to assess the efficacy of 10% (*w*/*v*) imidacloprid and 50% (*w*/*v*) permethrin combination to repel and kill adult mosquitoes on dogs in Thailand. *A. aegypti* mosquitoes were used as the model because they can be found worldwide, including in Thailand.

Materials and methods

Insecticide

A commercial preparation of 10% (*w*/*v*) imidacloprid and 50% (*w*/*v*) permethrin combination (Advantix®, Bayer AG, Leverkusen, Germany) was used in this study.

Experimental animals

Dogs Sixteen healthy dogs of both sexes (15–20 kg, local breed, more than 1 year old) were divided into two groups and subjected to this study. They were housed individually in cages at the laboratory animal facilities, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand under a 12-h light/dark cycle and were fed with commercial pellet feed with free access to food and clean water. Temperature was maintained at 28±2°C, and relative humidity was 80±5%. They did not receive any medicine for at least 30 days before the study and were never exposed to any insecticide and acaricide. This study was conducted under the approval of the Ethics Committee on Experimental Animal Usage and Animal Welfare, Faculty of Veterinary Science, Chulalongkorn University.

Mosquitoes Liverpool strain of *A. aegypti* mosquitoes of the 10th to 20th generations was used in this study. They were obtained from the University of Georgia and maintained at the Division of Parasitology, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University. All mosquitoes were maintained under controlled environmental conditions (27±1°C and 80±5% relative humidity) and fed with a 10% sucrose solution. Sucrose was withheld from mosquitoes 24 to 48 h before feeding on dogs.

Experimental design

The study design was in vivo on animal experiment. Eight dogs were subject to a treated group, and the other eight dogs were subject to an untreated control group. Two and a half milliliters of 10% (*w*/*v*) imidacloprid and 50% (*w*/*v*) permethrin combination was applied topically according to recommendations given by the manufacturer on each dog's skin of the treated group, and 2.5 ml of distilled water was applied topically on each dog's skin of the untreated control group. Each dog was sedated with 2 mg/kg of xylazine HCl and 0.04 mg/kg of atropine sulfate intramuscular injection and anesthetized with 10 mg/kg of pentobarbital sodium intravenous injection. Two cups of 50 female *A. aegypti* mosquitoes each were allowed to feed on each dog for each study day. Mosquitoes were kept in the plastic cup with nylon net on top. The cup was closely held onto the upper part of the hind leg and abdominal area of the dog. Mosquitoes were allowed to feed for 30 min. They were allowed to feed on days 10 and 3 before the insecticide combination application for the blood-feeding ability testing, and they were allowed to feed on days 3, 7, 14, 21, and 28 after the insecticide combination application for an efficacy testing.

Data analysis

The numbers of blood-fed, non-blood-fed, dead, and alive mosquitoes were counted. Feeding success rate was defined as the number of testing mosquitoes that took the blood meal from the dog, and mortality rate was defined as the number of testing mosquitoes that died after their attempts to feed on the dog, including both blood-fed and non-blood-fed mosquitoes. Feeding success rates and mortality rates were compared between treated group and untreated control group using paired *t* test and analysis of variance.

The IE of the combination was assessed by comparing the mean of alive mosquitoes in the treated group with the mean of alive mosquitoes in the untreated control group at a given time after treatment. The IE after exposure was calculated according to the following formula:

$$\text{Insecticide efficacy } (\%) = ((A - B/A) \times 100)$$

A=Mean of alive mosquitoes in the untreated control group

B=Mean of alive mosquitoes in the treated group

The repellent effect (RE) of the combination was assessed by comparing the mean of blood-fed mosquitoes in the treated group with the mean of blood-fed mosquitoes in the untreated control group at a given time after

treatment. The RE after exposure was calculated according to the following formula:

$$\text{Repellent effect}(\%) = ((A - B)/A) \times 100$$

A=Mean of blood-fed mosquitoes in the untreated control group

B=Mean of blood-fed mosquitoes in the treated group

Results

There were 16 dogs in this study. Eight dogs were in the treated group, and the other eight dogs were in the untreated control group. *A. aegypti* mosquitoes were tested for their blood-feeding ability on dogs on days 10 and 3 before the combination of 10% imidacloprid and 50% permethrin was applied onto the dog's skin of the treated group and the distilled water was applied onto the dog's skin of the untreated control group.

Blood-feeding success rates (mean±SE) of *A. aegypti* were shown in Table 1. The mean of the blood-feeding success rates of the mosquitoes that were exposed to the treated and untreated control dogs on day 10 before the combination application (BCA) were 78.0 and 71.4%, respectively, and on day 3 BCA were 50.4 and 52.5%, respectively. There was no significant difference of blood-feeding success rates between the mosquitoes that were exposed to the dogs allocated to the treatment or the control group on both tested days.

Blood-feeding success rates of the mosquitoes that were exposed to the treated dogs were 4.9 and 4.4% on days 3 and 7 post the combination application (PCA), respectively, and blood-feeding success rates increased to 6.3, 12.8, and 24.5% on days 14, 21, and 28 PCA, respectively. Blood-feeding success rates between the mosquitoes that were exposed to the treated and untreated control dogs on days 3, 7, 14, and 21 PCA were significantly different ($p<0.05$).

Mortality rates (mean±SE) of *A. aegypti* were shown in Table 2. All mosquitoes that attempted to feed on the treated dogs on day 3 PCA died, and mortality rates decreased to 97.1, 77.8, 40.4, and 2.1% on days 7, 14, 21, and 28 PCA, respectively. Mortality rates between the mosquitoes that were exposed to the treated and untreated control dogs on days 3, 7, 14, and 21 PCA were significantly different ($p<0.05$).

Percent RE of the insecticide combination was highest on day 3 PCA, which was 94.1%, and it decreased to 93, 92.8, 84.9, and 50.4% on days 7, 14, 21, and 28 PCA, respectively (Table 1). Percent IE of the insecticide combination was highest on day 3 PCA, which was 100%, and it decreased to 97, 77.8, 40.4, and 2.1% on days 7, 14, 21, and 28 PCA, respectively (Table 2).

Discussion

The objectives of this study were to evaluate the IE and RE of 10% (*w*/*v*) imidacloprid and 50% (*w*/*v*) permethrin against *A. aegypti* mosquitoes on dogs. Imidacloprid belongs to the neonicotinoid insecticide group, which the primary site of action is nicotinic acetylcholine receptors. It is taken up mainly by body contact and works by blocking the elements of the insect nervous system, which are more susceptible to toxic effects of imidacloprid than those of mammals. Imidacloprid causes tetanic muscle contraction in flea and also the dysfunction of nerve cells of the ganglia; however, it has no RE on flea larvae and adults (Mehlhorn et al. 2001). Permethrin also works as a contact insecticide, which causes nervous system toxicity and leads to the death of the insect.

Table 1 Mosquito blood-feeding success rates and repellent effect on *A. aegypti* on dogs treated with a combination containing 10% imidacloprid and 50% permethrin

Test day	Mosquito blood-feeding success rates (mean±SE)		Repellent effect (%)
	Treated dogs, n=8	Control dogs, n=8	
−10	78.0±7.7	71.4±10.1	–
−3	50.4±8.7	52.5±11.5	–
3	*4.9±3.1*	*83.4±7.1*	94.1
7	*4.4±2.6*	*63.1±11.5*	93.0
14	*6.3±2.3*	*88.0±4.7*	92.8
21	*12.8±4.4*	*84.8±7.2*	84.9
28	24.5±11.2	49.4±18.7	50.4

Italic numbers indicate significant differences between the mosquitoes that were exposed to the treated and untreated control groups ($p<0.05$)

Table 2 Mosquito mortality rates and insecticide efficacy on *A. aegypti* on dogs treated with a combination containing 10% imidacloprid and 50% permethrin

Test day	Mosquito mortality rates (mean±SE)		Insecticide efficacy (%)
	Treated dogs, n=8	Control dogs, n=8	
−10	0	0	–
−3	0	0	–
3	*100*	*0*	100.0
7	*97.1±1.5*	*2.0±0.9*	97.0
14	*77.8±7.7*	*0*	77.8
21	*40.4±10.6*	*0*	40.4
28	2.1±2.1	0	2.1

Italic numbers indicate significant differences between the mosquitoes that were exposed to the treated and control groups ($p<0.05$)

Imidacloprid and permethrin were combined for the synergistic property. The efficacies of this insecticide combination were previously examined on several acarids and insects (Epe et al. 2003; Fourie et al. 2006; Mehlhorn et al. 2003a; Mencke et al. 2003; Spencer et al. 2003; Blagburn et al. 2004). The criterion for assessment of the IE in this study was based on the comparison of the survival rate of *A. aegypti* between the treated and untreated control group. The repellency criterion in this study was based on the blood-feeding success rate of *A. aegypti* between the treated and untreated control group.

A. aegypti mosquitoes were chosen in this study because they play important roles in many pathogen transmission cycles in nature, particularly canine dirofilariosis (Tiawsirisup and Nithiuthai 2006). Because of the animal facility limitation of this study, *A. aegypti* mosquitoes were kept in the plastic cup with nylon net on top, and the cup was closely held onto the dog; thus, the result of this study might slightly differ from the results reported previously, where the dog was exposed to mosquitoes in a cage (Cruthers et al. 2003; Meyer et al. 2003).

After the mosquitoes were allowed to feed on the treated dogs, they did not stay away from the treated dog, but they tried to feed on the dogs. The mosquitoes, however, died after exposure to treated dogs within a couple of minutes, especially on the early days PCA. The similar scenario also happened in *Ixodes ricinus* ticks while trying to approach the treated dog's hair, but they moved back immediately after approaching the hair, without any further attempts. This effect has been specified as the hot-foot reaction (Mehlhorn et al. 2003b).

Feeding success rates in the control group were very high, with a range from 49.4 to 88.0%, and mortality rates in the control group were very low, with a range from 0 to 2%; these data validate the results obtained in the IE and RE studies. The IE in the study ranged from 100% on day 3 to 2.1% on day 28 PCA, and the RE in this study ranged from 94.1% on day 3 to 50.4% on day 28 PCA. This study showed that the RE was still high, which was 50.4%, but the IE was only 2.1% on day 28 PCA.

This study showed that blood-feeding success rates of the mosquitoes that were exposed to the treated dogs were 4.9, 4.4, 6.3, 12.8, and 24.5% on days 3, 7, 14, 21, and 28 PCA, respectively. Day 3 PCA was the first testing day of this study, so the onset of this insecticide combination cannot be indicated in this study. This insecticide combination could not completely prevent the mosquitoes from blood feeding on the dog; however, less than 5% of mosquitoes fed on the dog in the treated group such as on days 3 and 7 PCA. Some of these blood-fed mosquitoes, however, died after blood feeding on dogs in the treated group. All mosquitoes and more than 97% of the mosquitoes that were exposed to the treated dogs on days 3 and 7 PCA died. The study by Meyer et al. (2003) on 65% permethrin against *A. aegypti* on dog showed that the highest mortality of *A. aegypti* was only 90.9%, which is less than the mortality that was shown in this study, which used the combination of imidacloprid and permethrin; however, 65% permethrin caused the higher mortality of *A. aegypti*, which was 50.3% on day 28 PCA.

The blood-feeding success rate, which increased to 24.5%, and the mortality rate, which decreased to 2.1% on day 28 PCA, from this study indicated that the insecticide level in dogs of the treated group might be lower than protective level. These findings are similar to the study by Miro et al. (2007) on this insecticide combination against sand flies on dog, which also showed that this combination had a potent anti-feeding effect of more than 90% during the first 3 weeks of the trial only. It means an application of this insecticide combination should be repeated every 3–4 weeks to maintain the insecticide level in dogs as indicated.

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