The Efficacy of a Topically Applied Combination of Imidacloprid and Permethrin Against *Stomoxys calcitrans* on Dogs

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ABSTRACT

This laboratory study was undertaken to determine the efficacy of a combination of imidacloprid (10%) and permethrin (50%) applied topically as a spot-on, to prevent blood feeding by stable flies (*Stomoxys calcitrans*) on dogs. It was conducted in 3 replicates, and a total of 52 dogs were used. They were challenged with fasted, laboratory-reared *S. calcitrans* 1 day after treatment and at weekly intervals for 4 weeks thereafter. The efficacy of the imidacloprid/permethrin combination always exceeded 82% in preventing *S. calcitrans* from taking a blood meal on dogs during a period of 29 days after a single application, and the average efficacy over the entire period was 85.6%. In addition, more than 77% of flies that attempted to feed within the first 8 days after treatment died.

INTRODUCTION

The stable fly, *Stomoxys calcitrans*, as implied by its common name, is a pest of domesticated animals, although severe infestations have also been reported on wildlife.[1] The fly has a cosmopolitan distribution,[2] and both sexes are hematophagous.[3] Like many domestic flies, females lay their eggs in moist, rotting, or fermenting organic material, such as marine grass, straw, grain wastes, grass cuttings, and horse manure. One female lays up to 50 eggs at a time and can lay several batches in her lifetime. Eggs hatch in 2–5 days, depending on air and substrate temperature. Larvae mature in 11–20 days and are followed by a pupal period lasting 6–20 days. There is no proof that *S. calcitrans* is a biological vector of any human disease, but it does transmit Surra (*Trypanosoma evansi*) in horses and mules and equine infectious anemia (lentivirus) in horses and other single-hoofed animals. Its main importance, however, is that it is a vicious biter and blood-sucker.

The flies feed during the day and prefer warm, sunny, and windless weather. Daily activity appears to be bimodal with some activity during the morning and a peak in the late afternoon.[4] In urban and suburban

environments, *S. calcitrans* is often a serious nuisance problem on both humans and dogs. People are usually attacked on the lower parts of their legs, and the insertion of the fly's rigid proboscis in search of blood can be extremely painful. In dogs, the bites of stable flies cause continuous irritation and restlessness during the daylight hours. Because of this, the flies are frequently disturbed while feeding resulting in repeated attempts to feed. They attack the dorsal aspect of the muzzle, just posterior to the nose, and the ears in dogs, and the lesions they cause may vary from pinpoint hemorrhage to necrotic dermatitis on the tips of the ears.[5] In particular, stable fly bites give rise to so-called "ear bleeding," affecting mainly the tips of erect ears and the bent edge of floppy ears. This may lead to the development of allergies and to fly-bite dermatitis, a local ulcerative inflammation caused by bacterial infection.[6,7]

Imidacloprid is a neonicitinoid insecticide and has been developed for the control of various insects of agricultural and veterinary importance. Not only does a topically applied formulation have sustained insecticidal activity against adult fleas on dogs and cats, but it also is effective against flea larvae that come into contact with debris and hair from treated animals.[8] It is also effective against lice on dogs.[9] Permethrin is a synthetic pyrethroid that was first synthesized in 1973. It has a broad spectrum of activity against insects and acarines and is well known for its repellent efficacy against various arthropods. Permethrin and imidacloprid enhance each other's effect on the nervous system of the target species via separate and complementary activity along the axon and post-synaptic membrane, respectively.[10] The repellent and insecticidal effects of permethrin in combination with imidacloprid have been successfully tested against several mosquito species belonging to the genera *Culex* and *Aedes*.[11] Imidacloprid 10% and permethrin 50% have been combined in a novel formulation suitable for topical spot-on application on dogs (Advantix®, Bayer HealthCare AG, Germany), and the purpose of this investigation was to evaluate the immediate and sustained efficacy of this formulation in preventing *S. calcitrans* from taking blood meals on dogs.

MATERIALS AND METHODS

Three replicates, consisting of 20, 16, and 16 sub-adult to adult mixed breed dogs (mainly mongrels) of both sexes that had not been treated with an ectoparasiticide during the preceding 8 weeks, were selected for the study. The dogs were individually identified by means of implanted transponders and were housed separately, with no contact between animals possible. The study was performed in compliance with the guidelines for good clinical practice.[12]

On Day –7, all dogs in each replicate were challenged with a laboratory-reared strain of *S. calcitrans* and allocated to 2 equal groups. The allocation was done by randomization through minimization, using the Day –7 feeding success of the flies as a primary criterion. By random draw, the 2 groups in each replicate were then allocated to a treated group or an untreated control group.

Dogs in the treated groups weighing 10 kg or less were treated topically with the imidacloprid (10%)/permethrin (50%) combination at a dosage rate of 0.1 mL/kg body weight, applied as a single spot directly to the skin between the shoulder blades. Heavier dogs received 4 equally spaced spots between the shoulder blades and the base of the tail. Each dog was restrained for about 1 minute after application to allow the medication to spread.

Approximately 14-day old *S. calcitrans*, from which blood had been withheld and replaced with a 10% sucrose solution for 3 days before challenge, were fasted on the day prior to challenge. To facilitate challenge and subsequent assessment, the dogs were fasted overnight and sedated. The

sedated dogs were individually placed in fly-proof cages in environmentally controlled rooms (temperature = 25 ± 3°C; relative humidity = 40%–70%) and each dog was challenged with approximately 50 flies. After 30 minutes, live and dead (including moribund) flies were collected with an aspirator and counted. These flies were stored at below –20°C and subsequently squashed and the presence of blood in their intestines recorded. The experimental design is summarized in Table 1.

Table 1. Design of an Experiment to Evaluate the Efficacy of an Imidacloprid (10%)/Permethrin (50%) Combination in Preventing Stable Flies From Taking Blood Meals on Dogs.

Daily Activities			
Initial Fly Challenge	**Ranking and Allocation to Groups**	**Treatment**	**Fly Challenge and Efficacy Assessments**
–7	–3	0	+1, +8, +15, +18* +22, +29

Because of the large variation in individual values, efficacy in preventing blood feeding was calculated on each assessment day using geometric means, and the following equation was used:

Efficacy (%) = 100 × (mc – mt)/mc

mc = geometric mean number of flies that had taken a blood meal on dogs in the control group

mt = geometric mean number of flies that had taken a blood meal on dogs in the treated group

Some of the counts were zero and, consequently, geometric means were calculated after a natural logarithmic transformation of (count +1), and the corresponding arithmetic mean calculated. Thereafter a count of 1 was subtracted from the antilog of the mean value to obtain the geometric mean.

Since treatment killed substantial numbers of flies that attempted to feed, insecticidal efficacy based on mortality (dead and moribund flies) was also calculated using the same method and equation as above.

In order to consolidate the results over the 29-day periods of the studies, the area under curve (AUC) for the total number of flies that had taken a blood meal and the AUC for the number of live flies versus treatment day was calculated. Overall efficacy for the 29 days of the study was calculated from the geometric means of the AUC/(29–1) values in each group by the same equation for efficacy as described above.

The treated and untreated groups were also compared by an analysis of variance (ANOVA) with a treatment effect, based on the total number of flies that had fed on blood, and on the live fly counts on each assessment day, as well as on the AUC/(29–1) values. This ANOVA was performed on the original untransformed data.

RESULTS

The geometric mean numbers of flies that fed on untreated control dogs and on treated animals, as well as the efficacy in preventing blood feeding on each assessment day, and over the entire assessment period of 29 days, are graphically illustrated in Figure 1. Blood feeding by flies exposed to the untreated control groups of dogs was consistently high (≥10.7), and an average of 12.6 flies took a blood meal over the entire period of the study. The treated and control study groups differed significantly ($P < 0.001$) on all assessment days, as well as for the overall average [AUC/(29–1)]. Efficacy in preventing blood feeding was highest (90.2%) 1 day post-treatment and least (82.1%) 4 weeks after treatment.

The geometric mean numbers of flies that survived exposure to untreated and to treated dogs, as well as the % efficacy on each assessment day and over the entire assessment period of 29 days, are graphically illustrated in Figure 2. The survival of flies on untreated dogs was consistently high (≥21.4) with an average of 22.5 flies surviving over the entire assessment period. The efficacy of the imidacloprid/permethrin spot-on formulation in inducing mortality or

Figure 1. The efficacy of an imidacloprid (10%)/permethrin (50%) combination in preventing stable flies from taking a blood meal on untreated and treated dogs after 1 day and at weekly intervals after treatment and over the entire assessment period.

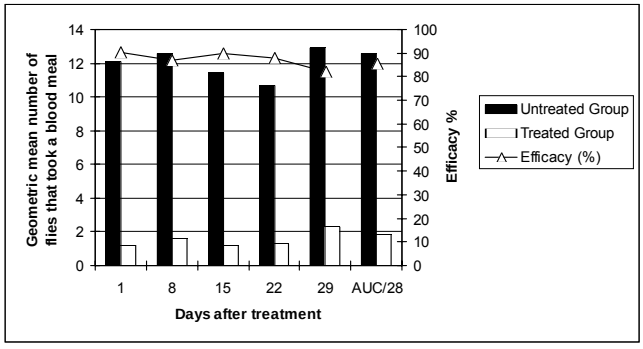
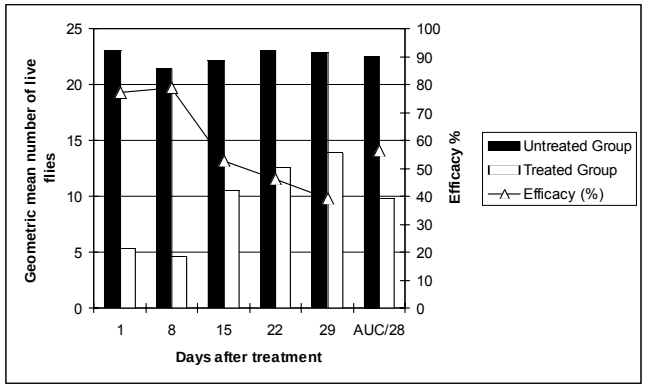


Figure 2. The geometric mean and average number of stable flies that survived exposure on various assessment days and over the entire assessment period, respectively, to untreated dogs and treated dogs with an imidacloprid (10%)/permethrin (50%) combination.



morbidity in exposed flies varied between 77.2% and 78.7% during the first 8 days after treatment. These values decreased to 39.2% on the last assessment day. The number of stable flies that survived exposure on the treated and control groups differed significantly ($P < 0.001$) on all assessment days, as well as over the entire assessment period [AUC/(29–1)].

DISCUSSION

Because stable flies are usually a problem on cattle and other species of farm animals, their propensity to bite dogs and other small animals has largely been overlooked. In 1 study, a daily mean of 314.1 stable flies were trapped in a greyhound kennel in Kansas, USA, over a 10-week period in the summer.[13] Their chief negative impact is not so much the removal of blood from their hosts, but rather the irritation and painful reactions that they cause and the possible mechanical transmission of pathogens. Their repeated attacks may also produce open wounds to which other flies are attracted. The tips of the ears are the most commonly affected site in dogs, and dermatitis with hemorrhagic, crusty lesions may develop.[6,7]

Problems with stable flies can be approached in a number of ways, all of which aim to prevent the flies from biting. This can be achieved by reducing breeding sites by disposal or management of manure, hay and grass clippings, repelling flies from animals, or by keeping animals indoors for the duration of stable fly activity. This advice is commonly displayed on veterinary clinics' Web sites. It is often not feasible for dog owners to keep their animals indoors since most dogs need regular walks several times per day and the flies are active during the whole photophase.

In the present set of studies, the topically applied formulation of imidacloprid/permethrin prevented more than 82% of *S. calcitrans* from feeding on dogs for 4 weeks after treatment. Average efficacy over the entire period of evaluation was 85.6%. In addition, more than 77% of flies exposed to treated dogs were killed up to 8 days after treatment. However, except in dog kennels, where the entire life cycle may take place, insecticidal efficacy is not the primary objective of treatment, for even though it is useful in reducing the number of stable flies, the chances of having a profound effect on their populations is low since they do not rely on dogs as their sole source of blood.

In a separate study against sand flies (*Phlebotomus papatasi*), the repellent (prevention of blood feeding) efficacy of the imidacloprid/permethrin spot-on was ≥72% up to 22 days post-treatment.[14] Repellent

efficacy against 2 other sand fly species (*Phlebotomus perniciosus* and *Lutzomyia longipalpis*) exceeded 90% for 3 weeks after treatment,[15] while blood feeding by the mosquito *Aedes aegypti* was reduced over a period of 4 weeks.[11]

Several repellents have been tested against *S. calcitrans* and are marketed as protection for companion animals.[16] Some of the earliest compounds were short-acting, requiring daily or twice-daily applications when the flies were particularly active. Others have to be applied at 2 to 3 day intervals, or whenever the owner feels that it is necessary. Moreover, several are contact repellents requiring that all body regions prone to fly attack must be treated. The advantages of the formulation of imidacloprid/permethrin used in the present set of studies are 1) its immediate and sustained efficacy against feeding of *S. calcitrans*, 2) that it is easily applied as a single or multiple spots along the back-line, and 3) that it also has an insecticidal effect on the fly. If applied regularly throughout the fly season, the risk of fly-bite dermatitis should be markedly reduced.

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