Evaluation of a Topical Solution Containing 65% Permethrin against the Sandfly (*Phlebotomus perniciosus*) in Dogs*

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**ABSTRACT**

A topical solution containing 65% permethrin (EXspot®, Schering-Plough Animal Health Corp., Union, NJ) was evaluated for repellency and insecticidal activity against the phlebotomine sandfly, *Phlebotomus perniciosus* (Diptera: Psychodidae). Four dogs were sedated and individually exposed to 100 female *P. perniciosus* inside a cage. Two of the dogs were treated on Day 0 with the permethrin solution by application of a 2-ml dose directly on the skin along the dorsal midline from the dorsal scapular area to the base of the tail. Two dogs remained untreated as controls. On Days –8, 0, 7, 14, 21, 28, 35, 49, 70, and 91, sandfly landings were recorded during the first 5 minutes of an insect exposure period and insect feeding and mortality rates were recorded after 1 hour of exposure. The number of sandfly landings was moderately decreased, and insect feeding rates decreased to zero by Day 7. From Day 14 through Day 28, the number of insect feedings was reduced from control values by greater than 90%. These findings indicate the permethrin spot-on provided a considerable repellent effect. Insect mortality rates remained very high (about 61%) during the 5 weeks after application, although insecticidal effects persisted for more than 7 weeks in total. These data suggest that monthly application of this topical solution of permethrin from the end of spring until the beginning of fall may protect dogs from the majority of sandfly bites. Further investigation with greater numbers of animals is warranted; however, results of this preliminary investigation indicate that a leishmaniasis control program that incorporates the strategic use of this product on dogs can be expected to decrease the number of infected animals and the incidence of the disease among the canine population.

**INTRODUCTION**

Leishmaniasis is a serious public health problem in many parts of the world. In the Mediterranean area, canine leishmaniasis is caused by the flagellated protozoa *Leishmania infantum* and is transmitted by the bite of female phlebotomine sandflies. *Phlebotomus perniciosus* is an important vector of this zoonotic
disease in this region and is active from the end of spring until the beginning of fall. Transmission can only take place during this period, although dogs may present signs at any time of the year due to the long incubation period of the disease. This creates not only a serious problem from a veterinary aspect but also a problem to human health, as dogs, which are believed to be the principal reservoir host of this disease, may also be the source of infection for humans. Each time a human is bitten by a sandfly that previously fed on an infected dog there is potential for *Leishmania* transmission.

Different strategies have been developed to attempt to control canine leishmaniasis, but until now none has been completely successful. As a long-term goal, researchers have attempted to develop a vaccine against the disease. Although some have shown promise, none has been satisfactory for commercial use. Chemotherapy in dogs with pentavalent antimonials is costly and generally ineffective. Alternative methods for use by dog owners continue to be the target of investigations for control of this vector-borne disease. One method is the use of insecticides and repellents to prevent the infective bite of the sandfly, thereby interrupting the transmission of leishmaniasis.

The efficacy of permethrin against hematophagous arthropods has previously been reported. Synthetic pyrethrins combine a long-lasting effect with low toxicity and new convenient formulations for use on dogs. This preliminary investigation was designed to examine the impact of a new topically applied spot-on 65% permethrin product (EXspot®, Schering-Plough Animal Health Corp., Union, NJ) for protecting dogs against bites of *P. perniciosus*.

**MATERIALS AND METHODS**

**Dogs**

Four beagles (two male and two female), 2 or 3 years of age, were used for the study. The animals were identified with numbered collars and were immunized against rabies, distemper, type II adenovirus, leptospirosis, and influenza during a 30-day adaptation period. At the end of this period, the four dogs were separated into pairs (one male and one female per pair) and treatments were randomly allocated to each pair. One pair was randomly assigned to be treated with the topical permethrin solution and the second group remained untreated as controls. Treated and control dogs were housed in separate kennels to avoid cross contamination. Dogs were fed commercial dry dog food daily and water was available free choice.

**Treatment**

A 65% topical permethrin solution was applied on Day 0 as a single dose. Each dog assigned to treatment with the permethrin solution weighed more than 15 kg, so a 2-ml dose was applied directly on the skin of each dog along the dorsal midline from the dorsal scapular area to the base of the tail. All dogs were observed hourly during the first 4 hours after application then daily for the remainder of the study for any signs of reactions to treatment. The two control dogs received no treatments.

**Sandfly Challenge**

*Phlebotomus perniciosus* were obtained from a laboratory colony established in 1987 from specimens captured in the province of Madrid, Spain. These specimens had been reared in an environmental chamber under controlled conditions of 27 ± 1°C, 90% to 100% relative humidity, and a photoperiod of 17 hours of light and 7 hours of darkness.

Each dog was exposed to 100 female sandflies on Days –8, 0, 7, 14, 21, 28, 35, 49, 70, and 91. Exposures on Days –8 and 0 were done to verify the challenge procedures and suitability of the sandfly colony for the evalua-
tion. Twenty-four hours prior to each challenge, 100 nine-day-old female sandflies were transferred into each of 10 gauze-covered plastic containers. On the initial challenge day (Day –8), each cage received a number corresponding to the dog identification number and this combination of cage and dog was not changed throughout the study. Feed was withheld from the dogs the day before each challenge. The room used for exposing the dogs to sandflies had a controlled constant temperature of 24 ± 2°C. Personnel who handled the dogs were different from those who handled the sandflies and always wore disposable gloves. All participating personnel were trained in the care and management of dogs.

Control dogs were handled before treated dogs to prevent accidental exposure of controls to the permethrin solution. To prepare for exposure to sandflies, each dog was sedated and immediately placed in a gauze-covered cage (75 × 50 × 50 cm), ensuring that all animals were placed in the same position. One hundred female and 20 to 25 male *P. perniciosus* sandflies were then released into each cage and left with the dogs for 1 hour. Two observers counted the number of sandfly landings on specific sites on each dog during the first 5 minutes of the 1-hour exposure period. The observers were unaware of the treatment groups, and the same sites were observed on each animal throughout the study. The arithmetic mean of counts of the two observers was calculated and recorded as the number of sandfly landings for the dog. Efficacy of the permethrin as a repellent was calculated using the following formula:

\[
\text{Percent reduction in sandfly landings} = \frac{\text{Mean landings on controls} - \text{Mean landings on treated dogs}}{\text{Mean landings on controls}} \times 100
\]

At the end of the challenge period, the dogs were removed from the cages and all sandflies were collected using a handheld vacuum. After each challenge, cages were thoroughly washed with detergent and acetone. From the sandfly populations recovered from each cage, numbers of engorged, surviving, and dead flies were determined and the following calculations were performed:

\[
\text{Percent reduction in blood-fed sandflies} = \frac{\text{Mean blood-fed flies from controls} - \text{Mean blood-fed flies from treated dogs}}{\text{Mean blood-fed flies from controls}} \times 100
\]

\[
\text{Percent sandfly mortality (based on exposure to 100 flies)} = \frac{\text{Mean live flies on controls} - \text{Mean live flies on treated dogs}}{\text{Mean live flies on controls}} \times 100
\]

**RESULTS**

**Repellent Effect**

The insects did not try to bite immediately after landing on dogs treated with the permethrin spot-on but appeared to move around on the hair without any particular direction, searching for an appropriate site to take a blood meal. After various periods of time, the insects ceased this behavior and flew away. This entire process was counted as one landing because even though contact was prolonged it involved a single landing. Data for insect landings are presented in Table 1. There was evidence of a repellent effect for the permethrin spot-on on Day 7, when there was a 78.5% reduction in sandfly landings on treated dogs. Evidence of a repellent effect based on the percentage reduction in sandfly landings was marginal after Day 7, and ranged from 0% to 42.1% from Day 14 to Day 91 (Table 1).
Insecticidal Effect

The number of engorged sandflies that fed on treated dogs was reduced by 100% on Day 7 and by 91.4% on Day 28, compared with numbers that fed on controls (Table 2). Efficacy of the permethrin spot-on declined from 79.8% on Day 35 to 42.2% on Day 91.

There was a notable increase in the number of dead sandflies from Day 7 to Day 49, demonstrating the insecticidal effect of the permethrin spot-on (Table 3). The percentage reduction in live flies on treated dogs was high-
est from Day 14 to Day 28, ranging from 66.8% to 74.1%. Insecticidal efficacy gradually waned, and there were similar numbers of live flies on both groups of dogs by Day 91.

There were no visible adverse responses to the permethrin spot-on at any time during the study.

**DISCUSSION**

Pretreatment challenges conducted on Days –8 and 0 confirmed that 100 female sandflies per dog was an adequate challenge population for this study. For each of the 10 challenges in the study, 400 *P. perniciosus* were used and it was necessary to produce nearly 8000 sandflies in the insectary to provide enough insects for the entire evaluation.

Counting the number of sandfly landings on each dog during the first 5 minutes of each assay turned out to be more difficult than expected due to the peculiar behavior of the flies. The sandflies moved around on the treated dogs for long periods, probing and searching for an appropriate place on the skin to take a blood meal. However, this behavior suggests that most flies had relatively long exposure to lethal doses of the insecticide. Although the stratum corneum of dogs is completely replaced after 1 month, there appears to be sufficient insecticide remaining in the skin (and possibly the fur next to the skin) to prevent sandflies from biting for 28 days after treatment as well as to cause the death of many parasites that come in contact with a treated dog.

The permethrin spot-on formulation appeared to disperse the active ingredient from the site of application over the body surface of the dog, impregnating the corneous layer of skin. However, there was little indication of any repellent effect of the material against *P. perniciosus* and only on Day 7 was there any observable difference between the two groups for number of insect landings on the dogs. This may be due to the fact that permethrin, as with many pyrethroids, has low vapor pressure and acts as a contact irritant to hematophagous insects. Nonetheless, the product was more than 90% effective in protecting against the

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*% Mortality = \( \frac{\text{Mean live flies on controls} - \text{Mean live flies on treated dogs}}{\text{Mean live flies on controls}} \times 100 \)
bites of the sandfly for at least 28 days after treatment.

The efficacy of permethrin against sandflies has been reported by others. However, the study reported here is the first evaluation of this insecticide as a topically applied product for dogs against the bite of a phlebotomine sandfly. Previous studies evaluated the level of protection against sandfly bites provided by deltamethrin-impregnated collars on dogs. The collars provided adequate protection for the majority of the sandfly season in areas tested; however, one disadvantage was that it took approximately 2 weeks or more after placement for collars to become fully effective. Results of the present study suggest that topical application of permethrin in this study may be more effective than the deltamethrin-impregnated collars; however, direct comparisons of efficacy and duration of protection have not been made between these two means of protection against sandflies in dogs.

Residual insecticides have been shown to be an effective tool for managing sandflies in the prevention of leishmaniasis. The idea of interrupting transmission of leishmaniasis by application of pyrethroid insecticides on dogs seems to be effective and attractive. The new pyrethroid formulations such as the topical permethrin solution in this study provide new and effective means for control of canine leishmaniasis. Further testing in a larger number of animals will be needed to confirm results in this preliminary investigation. The use of this treatment offers great potential for reducing sandfly populations and leishmaniasis; however, the rate of success will be heavily dependent on the proportion of the dog population treated as well as the size of the fly population present. Therefore, this form of treatment should be strategically integrated into control programs against canine leishmaniasis.

**CONCLUSION**

The results obtained in this preliminary study suggest that 65% permethrin provides effective protection for dogs against bites of sandflies and will also produce substantial mortality of these insects. Monthly application of EXspot® on the canine population during the period of high leishmaniasis transmission risk (early June to late September in the Mediterranean region) could be useful for prevention of canine leishmaniasis.

**REFERENCES**