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Efficacy of a new combination of fipronil and permethrin (Effitix®) against *Phlebotomus perniciosus* in dogs

M. Franc[a,*], E. Liénard[a], P. Jacquiet[a], S. Bonneau[b], C. Navarro[b], E. Bouhsira[a]

[a] Laboratoire de Parasitologie, Ecole Nationale Vétérinaire deToulouse, BP 87614, 23 Chemin des Capelles, 31076 Toulouse Cedex, France
[b] Laboratoire VIRBAC, 1ère Avenue, 2065 m L.I.D., 06516 Carros Cedex, France

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ABSTRACT

Two controlled clinical trials were carried out to assess the anti-feeding and adulticidal effects of a spot-on combining fipronil and permethrin (Effitix®, Virbac, Carros, France) against *Phlebotomus perniciosus* in dogs. The first study (Exp. A) was a dose-determination study in which 3 doses of permethrin (30 mg/kg, 60 mg/kg and 120 mg/kg) were compared. The second study (Exp. B) was an efficacy study using commercial dose of permethrin contained in Effitix® (the minimum dose of permethrin applied to dogs was 60 mg/kg). Twenty four and twelve Beagle dogs with equal sensitivity to sandflies were included in Exp. A and in Exp. B, respectively. Dogs were challenged with female sandflies (50 per dogs in Exp. A and 80 in Exp. B) for 60 ± 5 min on Days − 7, 1, 7, 14, 21 and 28 (Day 0 being treatment day). Counts and engorgement determination of dead and alive sandflies were performed after each exposure to treated and untreated dogs. Dead sandflies were also counted 24 h after exposure. In Exp. A, the repellency induced by an administration of 30 mg/kg of permethrin to dogs was above 91% for the first two weeks and then dropped to 82.2, 83.1 and 81.1% on Days 14, 21 and 28, respectively. For dogs receiving 60 mg/kg of permethrin, the repellency was a bit higher with 95.8, 97.6, 92.1, 91.4, and 86.8%, for Days 1, 7, 14, 21 and 28, respectively. The repellency induced by 120 mg/kg of permethrin was significantly higher than that induced by 60 mg/kg of permethrin on Day 14 only. In Exp. B the anti-feeding effect of the spot-on formulation was 94.1, 97.8, 96.3, 90.8 and 87% on Days 1, 7, 14, 21 and 28, respectively. The mortality effect was 98.9, 99.1, 99.8, 97.0 and 89.7% on Days 1, 7, 14, 21 and 28, respectively. At each challenge point, the mortality and anti-feeding effects on sandflies were significantly different between control and treatment group ($p < 0.05$). The results indicate that a monthly administration of this new combination of permethrin and fipronil could be used as an effective sandfly control strategy in dogs and therefore recommended for use in an integrated leishmaniosis prevention program.

1. Introduction

The phlebotomine sandfly *Phlebotomus perniciosus* is one of the main vectors of the agent of human and canine leishmaniosis in the Mediterranean Basin, *Leishmania infantum*. This zoonosis is spreading across Europe (Beugnet and Marié, 2009). Prevention of leishmaniosis in dogs can be achieved by using an integrative approach including an effective canine vaccine against *L. infantum* (Dantas-Torres, 2006; Solano-Gallego et al., 2011; Moreno et al., 2012; Oliva et al., 2014), associated with a topical registered veterinary product (i.e., synthetic pyrethroids, permethrin or deltamethrin) with a highly repellent effect against sandflies (Miró et al., 2007; Solano-Gallego et al., 2009; Maroli et al., 2010; Gramiccia, 2011; Beugnet and Franc, 2012). Preventing sandfly bites protects dogs from leishmaniosis and reduces the risk of human infection (Killick-Kendrick, 1999; Otranto et al., 2007; Quinnell and Courtenay, 2009). Several products have demonstrated their anti-feeding effect against sandflies such as a deltamethrin-impregnated collar (Killick-Kendrick et al., 1997; Reithinger et al., 2004; Franc and Bouhsira, 2009), a permethrin-pyriproxyfen spray (Molina et al., 2006), a permethrin-imidacloprid spot-on (Mencke et al., 2003; Miró et al., 2007; Otranto et al., 2007), a dinotefuran-permethrin-pyriproxyfen spot-on (Liénard et al., 2013), and recently, a fipronil-permethrin spot-on (Dumont et al., 2015).

This study was conducted to assess the anti-feeding and adulticidal effect of a new spot-on formulation combining fipronil and permethrin (Effitix®, Virbac, Carros, France) against *P. perniciosus* in dogs.

* Corresponding author. Fax: +33 5 61 19 39 71.
E-mail address: m.franc@envt.fr (M. Franc).

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2. Materials and methods

Two successive experiments were conducted at the Ecole Nationale Vétérinaire de Toulouse (ENVT), France. Experiment A (Exp. A) was a dose-determination study, whereas Experiment B (Exp. B) was an efficacy study using commercial dose of permethrin contained in Effitix®. Both studies were conducted according to Good Clinical Practice (GCP) as described in the International Cooperation and Harmonisation of Technical Requirements for registration of Veterinary Medicinal Product (VICH). Dogs were handled in accordance with the Animal Welfare and the study protocol was approved by the Ethics Committee of Midi-Pyrenees. All personnel involved with the collection of efficacy data were blinded for the treatment.

The studies were single-center, randomized, and controlled efficacy studies on four groups of six dogs in Exp. A and of two groups of six dogs in the Exp. B.

2.1. Dogs

Adult Beagle dogs were used in the experiments (Exp. A: 7 males and 17 females, 6–10 years of age, weighing 7.23–12.53 kg; Exp. B: 3 males and 9 females, 7–12 years old, weighing 7.85–11.19 kg) and had not been exposed to short-acting ectoparasiticides neither to long-acting ectoparasiticides for three months or one year, respectively, prior to treatment and they remained in good health throughout the study. They were housed in individual indoor cages in a controlled environment and had a 4-h daily access to a 2×4 m concrete run without contact with another dog. To avoid cross contamination, treated and untreated dogs were placed in two different exercise areas. Each dog was identified with the number of a subcutaneously implanted microchip. They were fed with a commercial dry dog food, getting a ration that maintained the animal in a healthy physical state. Dogs were maintained and handled with due regard for their welfare and were acclimatized to the caged environment for two weeks prior to treatment. They were observed daily for their general health conditions throughout the trials.

2.2. Sandfly exposures

The strain of sandflies used in this study was originated from Lisbon, Portugal, and had been maintained under laboratory conditions for 9 years at ENVT.

Sandfly exposures were performed one week prior to treatment (Day-7) for allocation purposes, and after treatment on Days 1, 7, 14, 21 and 28. The day before exposure, 50 (±2) female sandflies in Exp. A and 80 (±2) female sandflies in Exp. B were aspirated from their breeding cage with a vacuum pump and then placed in challenge nets with access to sugary-water-soaked cotton. Sandflies were fasted 2 h before exposure to dogs by removing the cottons from the cages.

Before exposure, dogs were sedated by intramuscular injections of a mixture of medetomidine (Dexdomitor®, Elanco Santé Animale, Lilly, Suresnes, France) and ketamine (Clorketam®, Laboratoire Vetoquinol S.A., Lure, France) and once the effects of anesthesia were visible, they received an intramuscular injection of diazepam (Valium®, Roche injectable, Neuilly s/ Seine, France) at a dose rate of 4 μg/kg, 9 mg/kg and 5 mg/dog, respectively, and then placed in individual infestation proof nets containing sandflies. The dose of the anesthetic was approximately calculated to immobilize dogs for 60 min. During infestation, treated and controlled dogs were placed in separated infestation rooms where temperature and relative humidity were maintained between 25 and 26 °C and between 58 and 72%, respectively. Cages and nests were thoroughly cleaned after each sandfly challenge.

After 60 ± 5 min of exposure, the dogs were carefully taken out of the net and examined for dead sandflies on their body, and then placed back in their cage. All live sandflies were aspirated from each challenge net using a vacuum pump and were recorded as live engorged or live non-engorged. All dead sandflies were collected, counted and recorded as dead non-engorged or dead engorged. On Days – 7, 1, 7, 14, 21 and 28, live sandflies recovered from individual animals at the end of exposure were placed in separate nets and kept in the experimental room. Sandflies were fed on sugar–water and checked for mortality after 24 h. Then, all remaining sandflies were discarded.

2.3. Allocation and treatment

2.3.1. Allocation

Dogs were allocated according to their individual pre-treatment sandfly engorgement status. They were ranked in descending order according to their individual number of engorged female sandflies. They were then introduced into blocks of two or four animals depending on the experiment. In Exp. A, blocks of 4 animals were formed and in each block, dogs were randomly allocated in 4 groups: control group (Group A) and groups treated with 6.70 mg/kg of fipronil combined with permethrin at the following doses: 30 mg/kg in Group B, 60 mg/kg in Group C and 120 mg/kg in Group D. In Exp. B, blocks of two animals were formed and in each block, dogs were randomly allocated to the control (Group 1) or treatment group (Group 2).

2.3.2. Treatment

In experiment A, the 6 dogs from the control group (Group A) remained untreated; dogs from Group B received 0.11 ml/kg of a 27.25% permethrin and 6.1% fipronil solution corresponding to a minimum dose of 30 mg/kg of permethrin and 6.70 mg/kg of fipronil; Group C received 0.11 ml/kg of a 57.5% permethrin and 6.1% fipronil solution corresponding to a minimum dose of 60 mg/kg of permethrin and 6.70 mg/kg of fipronil and Group D received 0.22 ml/kg of a 57.5% permethrin and 6.1% fipronil solution corresponding to a minimum dose of 120 mg/kg of permethrin and 6.70 mg/kg of fipronil.

In experiment B, the 6 dogs from the control group remained untreated, and the 6 dogs from the treatment group received on Day 0 a spot-on combination of permethrin and fipronil: one pipette of 1.1 ml (containing a 57.5% permethrin and 6.1% fipronil solution) for dogs weighing between 4.1 and 10.0 kg and one pipette of 2.2 ml (containing a 57.5% permethrin and 6.1% fipronil solution) for dogs weighing between 10.1 and 20 kg.

For all treated animals in both experiments, the formulation was applied according to manufacturer's instructions by parting the hair and applying the formulations on two spots directly on the skin: between the shoulder blades and on the lumbar area. All dogs were observed at 2 and 4 h after treatment for any adverse reactions to the product.

2.4. Data analysis

2.4.1. Anti-feeding effect

For each time point after exposure, the anti-feeding effect was calculated as described below:

$$\text{Anti} - \text{feedingeffect} = 100 \times \frac{\text{Ce} - \text{Te}}{\text{Ce}}$$

where Ce was the arithmetic mean of engorged female sandflies (live engorged and dead engorged) for the control group and Te was the arithmetic mean of the engorged female sandflies for the treatment group.