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Efficacy of dinotefuran, permethrin and pyriproxyfen combination spot-on against *Aedes aegypti* mosquitoes on dogs

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ABSTRACT

A spot-on formulation combining permethrin, dinotefuran and pyriproxyfen (Vectra 3D™ spot-on solution for dogs – one 10–25 kg pipette contains 196 mg dinotefuran, 1429 mg permethrin and 17 mg pyriproxyfen) was evaluated in adult Beagle dogs in a study designed to measure its efficacy to control *Aedes aegypti* (anti-feeding effect and mortality effect).

The trial was performed according to Animal Welfare and Good Clinical Practice.

Twelve dogs (five males and seven female, >3 years old, weighing 8.8–13.0 kg) were randomly allocated to treatment groups on pre-treatment mosquito counts: six dogs served as untreated controls, and six dogs were treated with the test formulation. Treatment consisted of applying a combination formulation to deliver at least 46.6 mg kg^{-1} permethrin, 6.40 mg kg^{-1} dinotefuran and 0.57 mg kg^{-1} pyriproxyfen. The combination is designed to control fleas, ticks, sand flies and mosquitoes. Each dog was infested with approximately 100 adult unfed *A. aegypti* once before treatment (day 6) then at 1, 7, 14, 21 and 28 days post-treatment. Counts and engorgement determination of dead and live mosquitoes were performed after 1 h exposure period. In the treated group (group A), the repellency effect of the product based on engorgement status (anti-feeding effect), was 91.5%, 94%, 94.7%, 94% and 87% at 1, 7, 14, 21 and 28 days post-treatment. Mortality effect or insecticidal efficacy calculated at the end of the 1-h exposure was almost identical when calculated 24 h after the 1-h exposure and remained above 93% until the end of the in-life phase. No adverse events were observed following treatment, including observations conducted 2, 4 and 24 h after the last dog was treated.

Aedes aegypti is a major vector of *Dirofilaria immitis* heartworm, the most serious mosquito-borne disease in dogs (Apperson et al., 1989; Russell et al., 2005; Genchi et al., 2009; Vezzani et al., 2011) and of *Dirofilaria repens* (Anyanwu et al., 2000). Dirofilariosis can be prevented by the use of anthelmintics such as moxidectin (Genchi et al., 2010), ivermectin, milbemycin oxime and selamectin (Blagburn et al., 2011). The application of a parasiticide having an anti-feeding effect on mosquitoes can be additional help preventing the risk of *Dirofilaria* transmission by infected mosquitoes (Haysaki and Saeki, 2009).

Controlling ectoparasites (fleas, ticks, sandflies and mosquitoes) on pets is a constant request from owners and a persistent issue for practitioners; as a consequence, new products combining different active ingredients are being

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developed. A formulation combining dinotefuran, permethrin and pyriproxyfen (Vectra 3D) was registered in the USA in 2007 and is indicated for the prevention and treatment of fleas, ticks, flies and mosquitoes on dogs.

Permethrin is a well-known insecticide used for years for the control of ectoparasites on companion animals and farm animals (Ross et al., 1997; Machida et al., 2008). Permethrin, as all of the pyrethroids, exerts its action on sodium voltage-dependent channels of the parasites. Pyrethroids modulate the conductance of the sodium ions in these channels by increasing the duration of their opening which leads to hyper-excitability and death of the parasite (Clark and Symington, 2012).

Dinotefuran is a third-generation rapid-acting nitroguanidine neonicotinoid insecticide exerting its action on a unique acetylcholine receptor present in the insect nerve synapse by mimicking the action of the neurotransmitter (Wakita et al., 2005). Pyriproxifen used in this combination targets the insect endocrine system by miming the activity of the insect juvenile hormone. It acts to stop the flea life cycle by preventing development of immature stages of fleas thereby arresting the development of flea eggs, flea larvae and pupae (Meola et al., 1996; Miller et al., 1999; Murphy et al., 2009).

This study was conducted to assess the efficacy of a permethrin–dinotefuran–pyriproxyfen spot-on formulation to repel and kill adult *A. aegypti* mosquitoes on dogs. The study was conducted in accordance with Animal Welfare and Good Clinical Practice.

1. Materials and methods

1.1. Dogs

Five males and seven female Beagle dogs (>3 years old, healthy, weighing 8.8–13.0 kg) from the École Nationale Vétérinaire de Toulouse (ENVT) were enrolled. Dogs had not been exposed to ectoparasiticides for 3 months prior to treatment and remained in good health throughout the study. Dogs were housed individually in cages indoors with controlled environmental conditions. Dogs were fed a commercial dry dog food ration calculated to maintain the animal in a healthy physical state. Water was available ad libitum through automatic lickers. No concurrent medication was given during the study. Dogs were managed similarly and with due regard for their well-being. Animals were handled in compliance with the relevant Institutional Animal Care and with the Regional Ethics Committee for animal experimentation.

The dogs were acclimated to study conditions for 14 days prior to treatment and were observed for general health conditions throughout the study. On day 7, each dog was challenged with 100 unfed adult female *A. aegypti*. They were ranked according to the number of *A. aegypti* biting into two groups of six (treated–untreated).

1.2. Mosquito maintenance and supply

A. aegypti (Liverpool strain) originally sourced from Milano were cultured at ENVT using a 5-week egg to adult cycle beginning July 2010. Mosquitoes were reared following Fortin and Slocombe (1981).

1.3. Treatment

Group A dogs remained untreated, group B dogs were treated with a permethrin, dinotefuran and pyriproxyfen combination spot-on 1.6 ml (dogs weighing between 4.1 and 10.0 kg) or 3.6 ml (dogs weighing between 10.1 and 25.0 kg). For all treated animals, formulation was applied by parting the hair and applying the spot-on directly to the skin: for dogs weighing less than 10.0 kg, a 1.6-ml pipette was used with half of the dose applied between the shoulder blades and half of the dose at the base of the tail, for dogs weighing more than 10.1 kg, a 3.6-ml pipette was used with one-third of the dose between the shoulder blades, one-third of the dose at the base of the tail, then one-third of the dose in the middle of the back (Vectra 3D™ spot-on solution for dogs – one 10–25 kg pipette contains 196 mg dinotefuran, 1429 mg permethrin and 17 mg pyriproxyfen). Treatment dosages were in the range of 69–135 mg kg^{-1} for permethrin, 9.5–18.5 mg kg^{-1} for dinotefuran and 0.84–1.64 mg kg^{-1} for pyriproxyfen.

1.4. Experimental procedure

The mosquitoes challenge assessment cages (60 cm × 40 cm × 50 cm) were constructed from mosquito netting mounted on a wooden frame and placed in environmentally controlled rooms. One hundred unfed female mosquitoes were placed in each of these 24 h before introducing the dogs which were sedated using ketamine[1] (9 mg kg^{-1}), medetomidine[2] (4 μg kg^{-1}) and diazepam[3] (5 mg dog^{-1}).

After 60 ± 5 min of exposure, the dogs were carefully taken out of the net and examined for any dead mosquito on their body, and then placed back in their cage. All live mosquitoes were aspirated from each challenge net using a vacuum pump and were recorded as live engorged or live non-engorged. All dead mosquitoes were collected, counted and recorded as dead non-engorged or dead engorged. During infestation, treated dogs and control dogs were placed in separated infestation rooms where temperature and relative humidity were maintained between 25 °C and 26 °C and between 58% and 72%, respectively. Cages and nets were thoroughly cleaned after each mosquito challenge.

On days −6, 1, 7, 14, 21 and 28, live mosquitoes recovered from individual animals at the end of exposure were placed in separate nets and kept in the experimental room. The mosquitoes were fed on sugar–water and checked for mortality after 24 h.

[1] Medetomidine, intramuscular (IM); Dexdomitor®, PFIZER Santé animale, 23-25 av Dr Lannelongue, 75668 Paris.

[2] Ketamine, IM; Clorketam®, Laboratoire VETOQUINOL S.A. 70204 Lure cedex.

[3] Diazepam, IM; Valium® Roche injectable, 52 Bd du parc, 92521 Neuilly s/ Seine cedex.

1.5. Data analysis

Anti-feeding effect: For each time point after exposure, the anti-feeding rate was evaluated for each group as described below and compared to the control group:

$$\text{Anti-feeding rate} = \frac{\text{Total number of unengorged mosquitoes}}{\text{Total number of recovered mosquitoes}}$$

Then the anti-feeding effect (expressed in percentage) was determined:

$$\text{Antifeeding effect} = 100 \times \frac{\text{anti-feeding rate in treated dogs} - \text{anti-feeding rate in untreated dogs}}{1 - \text{anti-feeding rate in untreated dogs}}$$

Mortality effect: For each time point after exposure, the mortality rate was evaluated for each group as described below and compared to the control group:

$$\text{Mortality rate} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of recovered mosquitoes}}$$

Then the mortality effect (expressed in percentage) was determined:

$$\text{Mortality effect} = 100 \times \frac{\text{mortality rate in treated dogs} - \text{mortality rate in untreated dogs}}{1 - \text{mortality rate in untreated dogs}}$$

The percentage of *A. aegypti* engorged females and live females in both groups were compared using a date-by-date analysis of variance followed by a non-parametric test of Kruskall–Wallis. Differences were considered significant at $P < 0.05$. The analyses were performed with Systat 9 software using percentage $\times 10$ as data.

2. Results

All dogs included in the study demonstrated adequate pre-treatment parasite holding ability. On day −7, the percentage of engorged female was, respectively, 87.6% for the treated group and 86.3% for the control group. All untreated animals maintained adequate engorged female level throughout the study (Fig. 1). Post-treatment percentage of engorged mosquitoes for the treated dogs was significantly lower than that for untreated control dogs (which ranged from 81% to 92% at all post-treatment evaluations – $P < 0.05$, Table 1). The treatment provided 91.5% anti-feeding efficacy on day 1 then ≥94% efficacy up to 3 weeks after treatment (day 21) and at the end of the study (day 28) 87% efficacy.

There was no significant statistical difference between both groups in mortality in females at day −6 and at day −5 meaning that pre-treatment, blood feeding on dogs from both groups did not lead to death of mosquitoes. Then, after 1 h of exposure and 24 h after each challenge point performed at day 1, day 7, day 14, day 21 and day 28, the difference in mortality of females *A. aegypti* between treated and controlled group was significant ($P < 0.05$).

The treatment had a mortality effect calculated at 1 h and at 24 h post-exposure above 93.0% and 93.4%, respectively, until the end of the animal phase. The maximum of mortality is obtained at day 7 with an efficacy of 100%, and then remained above 96.3% until day 21. The mortality effect calculated at 1 h and at 24 h post-treatment was approximately the same (Table 1).

3. Discussion

The objectives of this study were to evaluate the insecticidal effect and the repellent effect of a formulation with permethrin 36.08% (w/w), dinotefuran 4.95% (w/w) and pyriproxyfen 0.44% (w/w) combination against *A. aegypti* mosquitoes on dogs. This formulation provided excellent results with a good repellent effect – 94% and insecticidal effect – 96% for 3 weeks post-treatment falling to 87% in week 4. The treatment of dogs with permethrin–dinotefuran–pyriproxyfen formulation seems to offer better protection from *Aedes* mosquito bites than formulations of lower or similar dosage of permethrin combined with imidacloprid or with permethrin alone. Indeed, in similar trials performed with *A. aegypti* (Meyer et al., 2003; Tiawsirisup et al., 2007), insecticide efficacy for 65% permethrin alone ranged from 84% to 90.9% until day 21 and declined to 50.3% on day 28; insecticide efficacy for 50% permethrin combined with 10% imidacloprid ranged from 40.4% to 100% until day 21 and declined to 2.1% on day 28. The permethrin–dinotefuran–pyriproxyfen combination provided an insecticide efficacy between 93% (day 28) and 100% (day 7). The repellent effect obtained with permethrin alone ranged from 78% to 89.9% through day 21 and declined to 61.9% on day 28; for treatment combining imidacloprid and permethrin, the repellent effect ranged from 84.9% to 94.1% through day 21 and declined to 50.4% on day 28. The formulation tested in the current study offered a repellent effect ranging from 91.5% to 94.7% with minimal decline to 87% on day 28.

A. aegypti mosquitoes were chosen in this trial because of their medical importance in transmission of the yellow fever virus and other arboviruses and as a well-known vector of dirofilariosis which causes severe diseases and even death in dogs in many parts of the world (McCall et al., 2008). In addition, human beings can be affected by *D. immitis* and *D. repens* although they are dead-end hosts for these parasites (Estran et al., 2007; Genchi et al., 2011).

The experimental protocol proposed here tended to recreate natural infestation of dogs because mosquitoes were allowed to bite on their preferential sites (around eyes, around mouth and ventral part of the dog), as the full

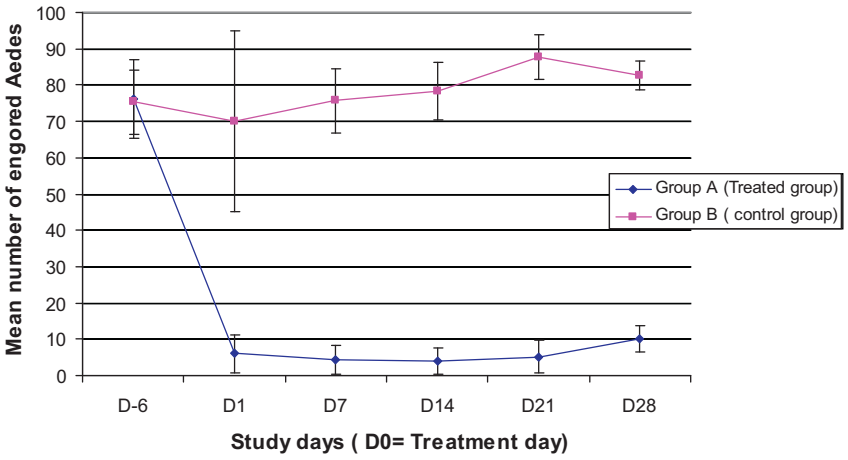

Fig. 1. Evolution of mean number of engorged *Aedes aegypti* obtained in both groups after 1 h exposure to dogs.

Table 1
Mortality and antifeeding effect.

	Day 1 (%)	Day 7 (%)	Day 14 (%)	Day 21 (%)	Day 28 (%)
Mortality effect					
1 h	99.0[a]	100.0	96.3	98.9	93.0
24 h	98.9[a]	100.0	96.5	99.1	93.4
Antifeeding effect (*P*-value)					
1 h	91.5(0.004)	94.0(0.004)	94.0(0.004)	94.0(0.004)	87.0(0.004)

[a] The mortality of A. aegypti in the control group during the 24 hours after the dog exposition explain the decrease of the mortality effect.

body of dogs was accessible. On the contrary, Tiawsirisup et al. (2007) presented *A. aegypti* trapped in plastic cups to dogs. The feeding rate they obtained in the control group ranged from 49.4% to 88% versus a feeding rate from 86.3% to 91.6% for the control group obtained in this current trial. These differences confirm the importance of sticking to natural conditions of infestation. Furthermore, mosquitoes were allowed to perform their entire blood meal without any disturbance or interruption as the dogs were asleep. Once the blood meal was performed, female mosquitoes left the dogs to lay on the side of the net which made it easier for their collection. Mosquitoes in contact with treated dogs died quickly after their exposure, especially on the early days post-treatment (cf. day 7 with insecticidal effect of 100%). This was confirmed by the insecticide effect which did not increase significantly 24 h after exposure.

Recently, an increasing number of veterinarians reported pet owners claiming that numerous parasiticide products were not efficient any more (Dryden et al., 2011) against ectoparasites of pets. Resistance is often cited. So the combination of new products or combination of well-known products with new chemistries which have not been associated yet could be a pertinent solution. Murphy et al. (2009) demonstrated that dinotefuran was more efficient than imidacloprid against *Ctenocephalides felis* on cats. In an assay against mosquitoes (Corbel et al., 2004), dinotefuran was less toxic than most of the commonly used insecticides (e.g., deltamethrin, carbosulfan and temephos); however, the efficacy of dinotefuran towards resistant mosquitoes was not strongly affected by the presence of common resistance mechanisms (kdr mutation and insensitive acetylcholinesterase). The difference of sites of action of dinotefuran, acetylcholine receptor versus sodium channels for pyrethroids, may explain the absence of cross-resistance with common parasiticides and makes dinotefuran a potential candidate for vector control in areas where mosquitoes may be resistant to common insecticides (Corbel et al., 2004).

Pyriproxifen, dinotefuran and permethrin are associated for the first time in a formulation. The interest of this association would be to broaden the spectrum of action to fleas and immature stages of fleas which could be very useful for dogs infested with fleas travelling in endemic areas of dirofilariosis, but this was not the aim for the current study which chose to focus on its efficacy towards *A. aegypti* mosquitoes only.

In conclusion, the combination of permethrin–dinotefuran–pyriproxyfen can be used on dogs to repel and kill *A. aegypti* mosquitoes reducing stress and annoyance caused by the bite of mosquitoes. More importantly, this combination may reduce the risk of heartworm transmission for animals leaving or travelling in *Dirofilaria* endemic areas. The application of this combination needs to be repeated every 3–4 weeks and it should not be seen as a substitute for heartworm prevention treatments.

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