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Repellent and insecticidal efficacy of a combination of dinotefuran, pyriproxyfen and permethrin (Vectra® 3D) against *Culex pipiens* in dogs

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ABSTRACT

Culex pipiens is an important vector of pathogens of substantial medical and veterinary importance such as *Dirofilaria immitis* and *Dirofilaria repens* or the West Nile Virus. The control of these mosquitoes is therefore essential to control the transmission of mosquito-borne agents to humans and animals. A combination of dinotefuran, permethrin and pyriproxyfen (Vectra® 3D) has already shown its efficacy against *Aedes aegypti*. The aim of this study was to confirm the efficacy of this combination in repelling and killing another species of mosquito, *Culex pipiens*, after a single topical application to dogs.

Twelve adult Beagle dogs with an equal receptivity to mosquitoes were included in the study and divided in two groups of six dogs: an untreated control group and a group treated with a combination containing 54 mg/mL dinotefuran + 4.84 mg/mL pyriproxyfen + 397 mg/mL permethrin (Vectra® 3D). All dogs were challenged with 80 *Culex pipiens* females for 90 ± 5 min on Days - 28, 1, 7, 14, 21 and 28. The treatment was applied once topically on Day 0. Count and engorgement determination of dead and live mosquitoes were performed after each exposure to treated and untreated dogs.

Compared to control dogs, the spot-on formulation provided a repellent efficacy (anti-feeding effect) against mosquitoes of 98.9%, 98.8%, 98.6%, 96.7% and 97.9% on Days 1, 7, 14, 21 and 28 respectively. There was a significant difference ($p \leq 0.05$) between the treated and controlled groups on every assessment day. The insecticidal efficacy on treated dogs at 90 min was 34.7%, 50.3%, 39.7%, 22.8% and 11.4% on Days 1, 7, 14, 21 and 28 respectively. There was a significant difference between the treated and controlled groups for live mosquitoes for all assessment days ($p < 0.05$).

A single topical application of a combination of dinotefuran, permethrin and pyriproxyfen showed a significant repellent effect (*i.e.* >96%) against *Culex pipiens* which lasted for 28 days. The results suggest that the Vectra® 3D spot-on solution could be used as an effective mosquito control strategy in dogs and is therefore recommended for use in a dirofilariosis prevention programme.

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1. Introduction

Culex pipiens mosquitoes play an important role in the transmission of pathogens of significant public and veterinary health importance. They are the primary vectors of the Saint Louis Encephalitis in the USA and the West Nile Virus (WNV, *Flaviviridae*, *Flavivirus*) in Europe and the USA (Farajollahi et al., 2011; Andreadis, 2012; Rizzoli et al., 2015). WNV is normally maintained through a bird-mosquito cycle but can be spread to a wide range of incidental hosts, such as horses and humans, where it causes severe neurological disorders (Mackenzie et al., 2004; Ahmetagić et al., 2015). *Culex pipiens* is one of the main vectors of the zoonotic filarial agents *Dirofilaria immitis* (heartworm) and *Dirofilaria repens* (subcutaneous filarial worm) (Licitra et al., 2010; Capelli et al., 2013; Mckay et al., 2013). *Dirofilaria repens* is responsible for subcutaneous dirofilariosis in dogs and is often associated with mild signs or subclinical infections, such as localized itching, mild skin lesions, or subcutaneous nodules in different body areas (Tarello, 2011). *Dirofilaria immitis* on the contrary causes severe disorders and even death in dogs in many parts of the world (McCall et al., 2008). In humans, ocular, subcutaneous and pulmonary forms of *D. immitis* and *D. repens* have been reported (McCall et al., 2008; Otranto et al., 2011a, 2011b; Kalogeropoulos et al., 2014).

Therefore it is important to establish an integrated control programme against these mosquitoes to prevent pathogen transmission to humans and animals in endemic areas. Integrated programmes include the use of mosquito repellents, and one current approach involves the use of existing molecules in combination (Tiawsirisup et al., 2007; Fankhauser et al., 2015; Franc et al., 2015). Pyrethroids such as permethrin and deltamethrin have a recognized efficacy against sandflies and mosquitoes, and have been used widely in companion animals (Meyer et al., 2003; Beugnet and Franc, 2012). Dinotefuran is a rapid-acting nitroguanidine furanicotinyl insecticide, representing a third generation of neonicotinoid. It exerts its action on an acetylcholine receptor present in the insect nerve synapse by mimicking the action of the neurotransmitter (Wakita et al., 2005). Pyriproxyfen is an analogue of the insect juvenile hormone. In mosquitoes, pyriproxyfen inhibits the metamorphosis by preventing the emergence of adults from pupae (Mulligan and Schaefer, 1990; Yapabandara et al., 2001). This juvenile hormone analogue has been widely used in the control of mosquitoes (*i.e. Aedes* sp., *Culex* sp., and *Anopheles* sp.) especially in malaria endemic areas (Harris et al., 2013; Mbare et al., 2014; Seccacini et al., 2014). A formulation combining dinotefuran, permethrin and pyriproxyfen (Vectra® 3D, Ceva, France) was launched in the USA in 2007 and in Europe in 2014. It is indicated for the prevention and treatment of fleas, ticks, flies sandflies and mosquitoes in dogs. This combination has already shown a good efficacy against the mosquito species *Aedes aegypti* (Franc et al., 2012).

The aim of the study was to evaluate the adulticidal and the repellent effects of Vectra® 3D on *Culex pipiens* in dogs.

2. Materials and methods

The study was conducted at the National Veterinary School of Toulouse (Ecole Nationale Vétérinaire de Toulouse, ENVT), France, according to the Good Clinical Practices (GCP study). The protocol was approved by the local Ethics Committee and dogs were handled in accordance with the animal welfare and the local Institutional Animal Care and Use Committee requirements (ethical clearance MP/14280312). All personnel involved with the collection of efficacy data were blinded to the treatment.

2.1. Dogs

Twelve Beagle dogs (six females and six males aged between 2 and 10 years and weighing between 9.01 and 12.95 kg) were included in the study and acclimatized to the trial environment for 38 days prior to treatment. They had not been exposed to short-acting ectoparasiticides for 3 months prior to the inclusion and had never been treated with any long-acting ectoparasiticides. They were housed in individual boxes and had a four-hour daily access to a 4×4 m concrete run where they could run and play with toys. Control and treated dogs were placed in two different exercise areas to avoid cross contamination. Dogs were observed daily for their general health conditions and remained in good health throughout the study.

On Day - 28 each dog was challenged with 80 unfed adult *Culex pipiens* females to assess their receptivity to mosquitoes. The number of engorged female mosquitoes was used for allocation to groups. Dogs were ranked in descending order of their individual mosquito's engorgement status and six blocks of two animals each were formed. Within blocks, dogs were randomly allocated to the treatment or control group.

2.2. Mosquito maintenance and supply

The *Culex pipiens* female mosquitoes used in this trial were reared at the ENVT laboratory and were 15-day old when used for infestation. The colony was originated from the Interdepartmental agreement for Mosquito Control on the Mediterranean coast (Entente Interdépartementale de Démoustication, EID), Montpellier, France, and was maintained at ENVT under laboratory conditions since 2001.

2.3. Treatment

The six dogs from the control group were not treated, and the other six received on Day 0 a topical combination of dinotefuran, pyriproxyfen and permethrin. They were treated with the commercial dose of the product based on their bodyweight (1.6 mL pipette for dogs weighing between 4.1 and 10.0 kg and 3.6 mL pipette for dogs weighing between 10.1 and 25 kg). Dogs were administered 0.23 ± 0.08 mL/kg BW of the solution corresponding to 12.67 mg/kg $\pm$ 4.08 of dinotefuran, 92.33 mg/kg $\pm$ 29.77 of permethrin and