

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



Emilie Bouhsira<sup>a</sup>, Emmanuel Liénard<sup>a</sup>, Faouzi Lyazrhi<sup>b</sup>, Philippe Jacquet<sup>a</sup>, Marie Varloud<sup>c</sup>, Audrey Deflandre<sup>c</sup>, Michel Franc<sup>a,\*</sup>

<sup>a</sup> Laboratoire de Parasitologie, Ecole Nationale Vétérinaire de Toulouse, BP, 87614, 23 chemin des capelles, 31076 Toulouse cedex, France

<sup>b</sup> École Vétérinaire de Toulouse, Unité pédagogique Biostatistique, 23 chemin des Capelles, F-31076 Toulouse, France

<sup>c</sup> Ceva Santé Animale S. A., 10 Avenue de la Ballastière, 33500 Libourne, France

### ARTICLE INFO

#### Article history:

Received 7 December 2015

Received in revised form 24 May 2016

Accepted 20 July 2016

Available online 21 July 2016

#### Keywords:

*Culex pipiens*

Mosquitoes

Repellency

Insecticidal effect

Dogs

Vectra® 3D

### 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 dirofilariasis prevention programme.

© 2016 The Authors. Published by Elsevier Ltd on behalf of World Federation of Parasitologists.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

\* Corresponding author.

E-mail addresses: [e.bouhsira@envt.fr](mailto:e.bouhsira@envt.fr) (E. Bouhsira), [e.lienard@envt.fr](mailto:e.lienard@envt.fr) (E. Liénard), [f.lyazrhi@envt.fr](mailto:f.lyazrhi@envt.fr) (F. Lyazrhi), [p.jacquet@envt.fr](mailto:p.jacquet@envt.fr) (P. Jacquet), [marie.varloud@ceva.com](mailto:marie.varloud@ceva.com) (M. Varloud), [audrey.deflandre@ceva.com](mailto:audrey.deflandre@ceva.com) (A. Deflandre), [m.franc@envt.fr](mailto:m.franc@envt.fr) (M. Franc).

## 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  $0.23 \pm 0.08$  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

1.13 mg/kg  $\pm$  0.36 of pyriproxyfen. The formulation was applied directly onto the skin according to the manufacturer's instructions: in two points for the 1.6 mL pipette (between shoulder blades and on the lumbar area) and in three points for the 3.6 mL pipette (between shoulder blades, on the dorsal midline and on the lumbar area). The formulation was provided by CEVA Animal Health.

All dogs were observed at 2 and 4 h after treatment for any adverse reaction to the product.

#### 2.4. Experimental procedure

The 12 dogs were infested with 80 ( $\pm$ 2) *C. pipiens* females on Days - 28, 1, 7, 14, 21 and 28. Before exposure, dogs were sedated by intramuscular injection of a mixture of medetomidine (Dexdomitor®, Pfizer Santé animale, Paris, France) and ketamine (Imalgène 1000®, Laboratoire Merial, Lyon, France) at a dose rate of 5  $\mu$ g/kg and 5 mg/kg respectively. Once sedated they received an intramuscular injection of diazepam (Valium®, Roche injectable, Neuilly s/ Seine, France) at a dose rate of 0.5 mg/kg, before they were placed in the individual nets containing mosquitoes. The dosage of the anaesthetic was calculated so as to immobilize dogs for 90 min and additional amount of the products were administered when needed.

Two days before exposure, mosquitoes were aspirated from their breeding cage with a vacuum pump and then placed in each challenge net. They were provided cottons soaked with water and had honey at their disposal. They were fasted 24 h before exposure to dogs by removing honey from each infestation net. The cottons soaked with water were removed at the introduction of the dogs. During exposure to mosquitoes, treated and controlled dogs were placed in two separated infestation rooms where temperature and relative humidity were maintained at 25 °C  $\pm$  3 °C and 60%  $\pm$  5% respectively. The light was turned off during the hour of exposure.

After 90  $\pm$  5 min of exposure, the dogs were carefully taken out of the net and examined for any dead mosquito on their body, and then were returned to their individual box. Live mosquitoes were aspirated from each net and were classified as live engorged or live non-engorged. The engorgement status was determined by observation of the abdomen distension and the presence of blood in it with the naked eye. All dead mosquitoes were collected and classified as dead engorged or dead non-engorged. Remaining live mosquitoes recovered from each dog were placed in separate nets and kept in the experimental room. They were checked for mortality 24 h after. Then, all remaining mosquitoes were discarded.

After each mosquito challenge, cages and nets were thoroughly cleaned.

#### 2.5. Data analysis

##### 2.5.1. Antifeeding effect or repellency

For each time point after exposure, the antifeeding effect was calculated as described below:

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

Ce stands for the arithmetic mean of engorged females mosquitoes (live engorged + dead engorged) for the controlled group and Te is the arithmetic mean of engorged female mosquitoes for the treated group.

##### 2.5.2. Mortality effect (or insecticidal effect)

For each time point after exposure, the mortality effect was evaluated for each group as described below:

$$\text{Mortality effect} = 100 \times \frac{\text{Cl}-\text{TI}}{\text{Cl}}$$

Cl stands for the arithmetic mean of live females mosquitoes (live engorged + live unengorged) for the controlled group and TI was the arithmetic mean of live female mosquitoes for the treated group.

The mortality effect was calculated at 90 min and 24 h post-exposure.

#### 2.6. Statistical analysis

The two groups were compared for the number of engorged females and the number of dead females at each challenge point. They were subjected to general linear model (GLM) procedures (split model) with the software Systat 12.0 (Systat, USA) to determine the effect of 3 factors: group (treated or control group), day of examination and dog (as random factor) and the interaction Group \* Day on the numbers of mosquitoes engorged or dead at 1.5 h and 24 h after their exposition to dogs. Means were separated with the Tukey's honest significance test (Systat 12.0). Insect capture data were transformed with square root prior to analysis and back transformed values are given in text and tables. Differences were considered significant at  $p < 0.05$ .

### 3. Results

The treatment was well tolerated and no adverse events were reported. According to the GLM results, the Group and Day effects were significant as well as associated interaction Group \* Day ( $p < 0.05$ ) but not the Dog effect ( $p > 0.05$ ).

### 3.1. Anti-feeding effect (or repellency) on mosquitoes

The 12 dogs included in the study demonstrated a good pre-treatment parasite holding ability or receptivity: above 50% of engorged females per dog (Fig. 1). On Day-28, the mean number of engorged female mosquitoes was 66.3 and 66 out of 80 for the control and treatment group respectively. All control dogs maintained an adequate number of engorged females (*i.e.* mean number above 55) throughout the study (Fig. 1).

The treatment had an anti-feeding effect above 98.6% for the first three expositions post-treatment (first two weeks) and remained above 96.7% until the end of the trial (Table 1). At each challenge point post-treatment the difference in the engorgement status of *C. pipiens* females between treated and controlled group was significant ( $p < 0.05$ ).

### 3.2. Mortality effect (or insecticidal effect) on mosquitoes

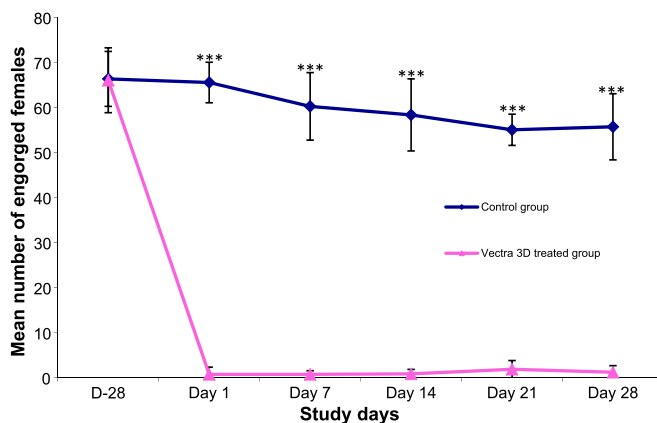
The mortality effects of the treatment calculated at 1.5 h and 24 h post-exposure to treated dogs are reported in Table 1. The mortality effect observed at 1.5 h ranged between 34.7% and 50.3% for the first two weeks after treatment and then dropped to values ranging between 11.4% and 22.8% for the last two expositions. The mortality effect of the formulation did not increase significantly within the 24 h post-exposure (Table 1). At each challenge point, there was a significant difference ( $p < 0.05$ ) in the number of dead mosquitoes found at 1.5 h and 24 h of exposure between the treated and controlled group.

## 4. Discussion

The results of this study showed that a single topical application of a combination of dinotefuran 4.95% (w/w), pyriproxyfen 0.44% (w/w) and permethrin 36.08% (w/w) (Vectra® 3D, Ceva, Libourne, France) provided an excellent feeding inhibition of *Culex pipiens* mosquitoes which lasted for four weeks. The combination provided a strong immediate effect with a 98.9% repellency reached 24 h after treatment. This efficacy remained above 96.7% for 28 days.

Recent studies reported the efficacy of other combinations containing permethrin combined with fipronil (Effitix®, Frontline Tri-Act®/Frontect®) on the same target, *Culex pipiens* (Fankhauser et al., 2015; Franc et al., 2015). The feeding inhibition provided by Vectra® 3D was similar or even better to the one obtained with these both new spot-on formulations containing approximately the same dose of permethrin. The repellency of Effitix® against *C. pipiens* was 100% (Day 1), 99.5% (Day 7), 97.7% (Day 14), 98.3% (Day 21) and 96.7% (Day 28) (Franc et al., 2015) while the efficacy of Frontline Tri-Act®/Frontect® was a bit lower with 99.4% (Day 1), 98.9% (Day 7), 94.7% (Day 14), 91.7% (Day 21) and 90.4% (Day 28) (Fankhauser et al., 2015).

The efficacy of Vectra® 3D was investigated in a previous study on another mosquito strain, *Aedes aegypti*, under the same laboratory conditions (Franc et al., 2012). The repellency reached 91.5% on Day 1, 94% on Days, 7, 14 and 21 and 87% on Day 28, which was close to the repellency obtained with *C. pipiens*. However differences in insecticidal efficacies were observed between these two mosquito species. Vectra® 3D presented an insecticidal efficacy against *C. pipiens* remaining between 50.3% (Day 7) and 11.4% (Day 28). On the contrary, the insecticidal efficacy against *A. aegypti* was significantly higher (*i.e.* >93%) despite a shorter exposure time to treated dogs (60 versus 90 min) (Franc et al., 2012). Interestingly, similar discrepancies were obtained by Fankhauser et al. (2015) with permethrin combined with fipronil (Frontline Tri-Act®/Frontect®) against *C. pipiens* and *A. aegypti*. The authors obtained an insecticidal efficacy against *C. pipiens* of 77.9%, 92.1%, 34.7%, 38.5% and 26.9% on Days 2, 8, 15, 22 and 29 while the insecticidal efficacy obtained against *A. aegypti* was 99.8%, 93.8%, 69.5%, 77% and 20.9% on Days 1, 7, 14, 21 and 28 respectively. They suggested that the repellent effect of permethrin combined with fipronil was stronger on *C. pipiens* than on *A. aegypti* therefore preventing them from landing on the treated dogs, thus limiting the contact with the



**Fig. 1.** Mean number of engorged *Culex pipiens* females after 90 min exposure to control and treated dogs. Dogs from treated group received on Day 0 a spot-on combination of dinotefuran, permethrin and pyriproxyfen (Vectra® 3D), dogs from controlled group remained untreated. All dogs were weekly challenged with 80 *Culex pipiens* females. At each challenge point after treatment, the differences between groups were statistically significant ( $p < 0.05$ ). \*\*\* represents statistical differences at  $p < 0.0001$ .

**Table 1**Mortality and anti-feeding effect of a dinotefuran, permethrin and pyriproxyfen spot-on combination (Vectra® 3D) against *Culex pipiens* females on dogs.

		Day 1	Day 7	Day 14	Day 21	Day 28
Anti-feeding effect (%) (95%CI)	1.5 h	98.9 (93–100)	98.8 (92.6–100)	98.6 (92–100)	96.7 (86.6–100)	97.9 (89.8–100)
Mortality effect (%) (95%CI)	1.5 h	34.7 (7.8–61.6)	50.3 (22–78.6)	39.7 (12–67.4)	22.8 (0–46.5)	11.4 (0–29.4)
	24 h	40.2 (12.5–67.9)	51.0 (22.7–79.3)	41.2 (13.4–69)	22.3 (0–45.9)	34.8 (7.8–61.8)

insecticidal molecules. However, there is currently no clear explanation on these differences of susceptibility between these two mosquito species. Lupi et al. (2013) investigated the literature review of repellency studies conducted on different products (DEET, Icaridin, Insect Repellent and plant-derived products (Citriodora or para menthane-3,8-diol)) against *Anopheles* sp., *Culex* sp. and *Aedes* sp. regarding human skin. All the tested molecules provided a better repellency (anti-feeding effect) on *Culex* sp. than on *Anopheles* sp. or *Aedes* sp. The authors reported also that *Aedes* sp. demonstrated a more aggressive biting behaviour than *Culex* sp. This aggressive behaviour has also been observed in our experimental conditions in which dogs were infested with mosquitoes. Once in contact with dogs, *A. albopictus* or *A. aegypti* land and bite more promptly than *C. pipiens* that take longer before tempting to feed (authors' personal observations). This behaviour could explain that the contact duration of *Aedes* sp. mosquitoes with the treated skin is increased, therefore improving the insecticidal efficacy (or mortality effect) of the molecule. A further reason could be related to the different feeding preferences of *C. pipiens* in comparison to *Aedes* sp: *C. pipiens* feeds preferentially on avian hosts while *Aedes*, especially *A. albopictus*, tends to feed predominantly on mammals including humans and pets (Gomes et al., 2013; Faraji et al., 2014; Valerio et al., 2010). However, further behavioural studies would be necessary to investigate this difference in host attractancy under laboratory conditions. These observations would bring significant inputs to the design of repellency studies using mosquitoes, as the exposure duration to the hosts must be long enough to allow a good feeding rate on control animals.

Vectra® 3D is currently the only ectoparasiticide combination for dogs containing dinotefuran. This molecule has a low affinity for binding sites of other neonicotinoid insecticides and has no cross-resistance with common parasiticides, such as permethrin (Wakita et al., 2005). Therefore, dinotefuran has been proposed as a potential candidate for vector control in areas with resistance of mosquitoes to other insecticides (Corbel et al., 2004).

## 5. Conclusion

A combination of dinotefuran, pyriproxyfen and permethrin provides a good protection (*i.e.* a repellency above 96.7%) against *Culex pipiens* in dogs for one month following a single topical application. Therefore this treatment could be included in an integrated prevention program against heartworm disease for dogs living in or travelling to dirofilariasis endemic areas. However, it should not be seen as a substitute for heartworm prevention treatments.

## Competing interests

The work reported here was partially funded by a grant from Ceva Santé Animale (CEVA-ST-CLS-C661-1203), Libourne, France. Two authors (MV and AD) are currently employees of Ceva Santé Animale, and assisted with the study design and the review of the manuscript. However, there were no conflicts of interests that may have biased the work reported in this paper.

## Acknowledgments

The authors are grateful to Sonia Gounaud and Martine Roques for their help with the mosquito colony maintenance and the handling of dogs.

## References

- Ahmetagić, S., Petković, J., Hukić, M., Smriko-Nuhanović, A., Piljić, D., 2015. Human West Nile virus infection in Bosnia and Herzegovina. *Med. Glas.* 12, 47–51.
- Andreadis, T.G., 2012. The contribution of *Culex pipiens* complex mosquitoes to transmission and persistence of West Nile virus in North America. *J. Am. Mosq. Control Assoc.* 28, 137–151.
- Beugnet, F., Franc, M., 2012. Insecticide and acaricide molecules and/or combinations to prevent pet infestation by ectoparasites. *Trends Parasitol.* 28, 267–279.
- Capelli, G., Frangipane di Regalbano, A., Simonato, G., Cassini, R., Cazzini, S., Cancrini, G., Otranto, D., Pietrobelli, M., 2013. Risk of canine and human exposure to *Dirofilaria immitis* infected mosquitoes in endemic areas of Italy. *Parasit Vectors* 6, 60.
- Corbel, V., Duchon, S., Zaim, M., Hougard, J.M., 2004. Dinotefuran: a potential neonicotinoid insecticide against resistant mosquitoes. *J. Med. Entomol.* 41, 712–717.
- Fankhauser, B., Dumont, P., Hunter, J.S., McCall, J.W., Kaufmann, C., Mathis, A., Young, D.R., Carroll, S.P., McCall, S., Chester, S., Soll, M.D., 2015. Repellent and insecticidal efficacy of a new combination of fipronil and permethrin against three mosquito species (*Aedes albopictus*, *Aedes aegypti* and *Culex pipiens*) on dogs. *Parasit Vectors* 8, 64.
- Faraji, A., Egizi, A., Fonseca, D.M., Unlu, I., Crepeau, T., Healy, S.P., Gaugler, R., 2014. Comparative host feeding patterns of the Asian tiger mosquito, *Aedes albopictus*, in urban and suburban Northeastern USA and implications for disease transmission. *PLoS Negl. Trop. Dis.* 8, e3037.
- Farajollahi, A., Fonseca, D.M., Kramer, L.D., Marm Kilpatrick, A., 2011. "Bird biting" mosquitoes and human disease: a review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect. Genet. Evol.* 11, 1577–1585.



- Franc, M., Genchi, C., Bouhsira, E., Warin, S., Kaltsatos, V., Baduel, L., Genchi, M., 2012. Efficacy of dinotefuran, permethrin and pyriproxyfen combination spot-on against *Aedes aegypti* mosquitoes on dogs. *Vet. Parasitol.* 189, 333–337.
- Franc, M., Lienard, E., Jacquet, P., Bonneau, S., Bouhsira, E., 2015. Efficacy of fipronil combined with permethrin commercial spot on (Effitix®) preventing *Culex pipiens* from feeding on dogs. *Parasitol. Res.* 114, 2093–2097.
- Gomes, B., Sousa, C.A., Vicente, J.L., Pinho, L., Calderón, I., Arez, E., Almeida, A.P., Donnelly, M.J., Pinto, J., 2013. Feeding patterns of *molestus* and *pipiens* forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. *Parasit Vectors* 6, 93.
- Harris, C., Lwetoijera, D.W., Dongus, S., Matowo, N.S., Lorenz, L.M., Devine, G.J., Majambere, S., 2013. Sterilising effects of pyriproxyfen on *Anopheles arabiensis* and its potential use in malaria control. *Parasit Vectors* 6, 144.
- Kalogeropoulos, C.D., Stefanidou, M.I., Gorgoli, K.E., Papadopoulou, C.V., Pappa, C.N., Paschidis, C.A., 2014. Ocular dirofilariasis: a case series of 8 patients. *Middle East Afr. J. Ophthalmol.* 21, 312–316.
- Licitra, B., Chambers, E.W., Kelly, R., Burkot, T.R., 2010. Detection of *Dirofilaria immitis* (Nematoda: Filarioidea) by polymerase chain reaction in *Aedes albopictus*, *Anopheles punctipennis*, and *Anopheles crucians* (Diptera: Culicidae) from Georgia, USA. *J. Med. Entomol.* 47, 634–638.
- Lupi, E., Hatz, C., Schlagenhauf, P., 2013. The efficacy of repellents against *Aedes*, *Anopheles*, *Culex* and *Ixodes* spp. - a literature review. *Travel Med. Infect. Dis.* 11, 374–411.
- Mackenzie, J.S., Gubler, D.J., Petersen, L.R., 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat. Med.* 10 (12), 98–109.
- Mbare, O., Lindsay, S.W., Fillinger, U., 2014. Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae sensu stricto* and *Culex quinquefasciatus*. *Parasit Vectors* 7, 280.
- McCall, J.W., Genchi, C., Kramer, L.H., Guerrero, J., Venco, L., 2008. Heartworm disease in animals and humans. *Adv. Parasitol.* 66, 193–285.
- Mckay, T., Bianco, T., Rhodes, L., Barnett, S., 2013. Prevalence of *Dirofilaria immitis* (Nematoda: Filarioidea) in mosquitoes from northeast Arkansas, the United States. *J. Med. Entomol.* 50, 871–878.
- Meyer, J.A., Disch, D., Cruthers, L.R., Slone, R.L., Endris, R.G., 2003. Repellency and efficacy of a 65% permethrin spot-on formulation for dogs against *Aedes aegypti* (Diptera: Culicidae) mosquitoes. *Vet. Ther.* 4, 135–144.
- Mulligan, F.S., Schaefer, C.H., 1990. Efficacy of a juvenile hormone mimic, pyriproxyfen (S-31183) for mosquito control in dairy waste water lagoons. *J. Am. Mosq. Control Assoc.* 6, 89–92.
- Otranto, D., Brianti, E., Gaglio, G., Dantas-Torres, F., Azzaro, S., Giannetto, S., 2011a. Human ocular infection with *Dirofilaria repens* (Railliet and Henry, 1911) in an area endemic for canine dirofilariasis. *Am.J.Trop. Med. Hyg.* 46, 1002–1004.
- Otranto, D., Diniz, D.G., Dantas-Torres, F., Casiraghi, M., de Almeida, I.N., de Almeida, L.N., dos Santos, J.N., Furtado, A.P., de Almeida Sobrinho, E.F., Bain, O., 2011b. Human intraocular filariasis caused by *Dirofilaria* sp. nematode, Brazil. *Emerg. Infect. Dis.* 17, 863–866.
- Rizzoli, A., Bolzoni, L., Chadwick, E.A., Capelli, G., Montarsi, F., Grisenti, M., De la Puente, J.M., Muñoz, J., Figuerola, J., Soriguer, R., Anfora, G., Di Luca, M., Rosà, R., 2015. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. *Parasit Vectors* 8, 213.
- Seccacini, E., Juan, L., Zerba, E., Licastro, S., 2014. *Aedes aegypti* (Diptera: Culicidae): evaluation of natural long-lasting materials containing pyriproxyfen to improve control strategies. *Parasitol. Res.* 113, 3355–3360.
- Tarello, W., 2011. Clinical aspects of dermatitis associated with *Dirofilaria repens* in pets: a review of 100 canine and 31 feline cases (1990–2010) and a report of a new clinic case imported from Italy to Dubai. *J. Parasitol. Res.* 2011, 578385.
- Tiawsirisup, S., Nithiuthai, S., Kaewthamasorn, M., 2007. Repellent and adulticide efficacy of a combination containing 10% imidacloprid and 50% permethrin against *Aedes aegypti* mosquitoes on dogs. *Parasitol. Res.* 1013, 527–531.
- Valerio, L., Marini, F., Bongiorno, G., Facchinelli, L., Pombi, M., Caputo, B., Maroli, M., Della Torre, A., 2010. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in urban and rural contexts within Rome province, Italy. *Vector Borne Zoonotic Dis.* 10, 291–294.
- Wakita, T., Yasui, N., Yamada, E., Kishi, D., 2005. Development of a novel insecticide, dinotefuran. *J. Pestic. Sci.* 30, 122–123.
- Yapabandara, A.M., Curtis, C.F., Wickramasinghe, M.B., Fernando, W.P., 2001. Control of malaria vectors with the insect growth regulator pyriproxyfen in a gem-mining area in Sri Lanka. *Acta Trop.* 80, 265–276.