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Anti-feeding and insecticidal efficacy of a topical administration of dinotefuran–pyriproxyfen–permethrin spot-on (Vectra® 3D) on mice against *Stegomyia albopicta* (= *Aedes albopictus*)

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Abstract. An ectoparasiticide combining three active ingredients [dinotefuran, permethrin and pyriproxyfen (DPP)] was used in mice in an experiment designed to evaluate its anti-feeding and insecticidal efficacy against *Stegomyia albopicta* (= *Aedes albopictus*) (Diptera: Culicidae) mosquitoes. Twenty-two adult mice were randomly allocated into two groups consisting of an untreated control group and a DPP-treated group. Mice were exposed individually for 1 h to a mean \pm standard deviation of 27 ± 2 starved female mosquitoes on days 1, 7, 14, 21 and 28 post-treatment. At the end of the exposure (1 h), mosquitoes were assessed for immediate survival and engorgement status. Additionally, live mosquitoes in both groups were incubated separately and observed for mortality at 24 h after the end of the exposure. The anti-feeding efficacy of DPP after the 1-h exposure period was 99.2, 100, 98.0, 89.3 and 87.4% at 1, 7, 14, 21 and 28 days, respectively. Levels of insecticidal efficacy evaluated at 1 h and 24 h after exposure on days 1, 7, 14, 21 and 28 were 36.7, 28.9, 30.8, 23.1 and 11.9%, and 68.4, 45.0, 43.3, 37.9 and 19.9%, respectively. Based on the mouse model, the present study demonstrates that the DPP combination has significant anti-feeding and insecticidal efficacy against *S. albopicta* for at least 4 weeks.

Key words. *Stegomyia albopicta* (= *Aedes albopictus*), ectoparasiticide, efficacy, mosquito-borne diseases, mouse model.

Introduction

The global range of the Asian tiger mosquito, *Stegomyia albopicta* (= *Aedes albopictus*) (Skuse), has undergone a dramatic expansion over the past 25 years into numerous countries outside Asia, particularly in the American, Pacific, European, Mediterranean and African regions (Gubler, 2003; Faraji *et al.*, 2014). It is now considered one of the most invasive mosquito

species in the world. *Stegomyia albopicta* is implicated as a competent vector of many emerging and re-emerging human arboviruses such as Chikungunya, Zika, dengue and yellow fever (Gratz, 2004; Delatte *et al.*, 2008; Lambrechts *et al.*, 2010). *Stegomyia albopicta* is also involved in the transmission of *Dirofilaria immitis* and *Dirofilaria repens*, parasitic nematodes that cause, respectively, heartworm disease and subcutaneous dirofilariosis in dogs and cats (Cancrini *et al.* 2003a, 2003b; Gratz,

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2004; Licitra *et al.*, 2010). Both parasites can also be transmitted to humans through the bite of an infected mosquito and will cause pulmonary and/or subcutaneous or ocular dirofilariosis (Foissac *et al.*, 2013; Benzaquen *et al.*, 2015). The dirofilarioses represent major veterinary and public health concerns and continue to be diagnosed in several regions of the world, which qualifies them as emerging zoonoses (Simon *et al.*, 2012; Dantas-Torres & Otranto, 2013).

Female *S. albopicta* are characterized by a wide range of host feeding preferences. They feed predominantly on mammalian hosts (83%), including humans (24%), cats (21%) and dogs (14%). These mosquitoes are also opportunistic feeders and can take a significant proportion of bloodmeals from avian and amphibian hosts (Richards *et al.*, 2006; Kamgang *et al.*, 2012). Therefore, the feeding plasticity of *S. albopicta* may increase the risk for transmission of zoonotic pathogens from wildlife or domestic animals to humans (Gubler, 2003; Delatte *et al.*, 2010).

Protecting dogs from mosquito bites is essential to prevent mosquito-borne pathogen transmission. It also reduces the risk for transmission of pathogens of zoonotic importance. Given the lack of vaccines against numerous vector-borne diseases, the best means of reducing mosquito–pathogen transmission is to avoid host–vector contact. Strategies adopted to achieve this objective rely on the use of individual protection involving the regular application of insecticides and the reduction of mosquito densities by the control of mosquito fauna using adulticidal and larvicidal products. In dogs, insecticidal products with repellent properties usually offer protection against mosquitoes, sandflies, fleas and ticks, and their effects are expected to last over 1 month after administration. Most act by contact and repellency is assessed through anti-feeding efficacy. It is also important to assess the survival of mosquitoes after exposure in order to evaluate the potential for reducing the spread of parasites.

Recently, a formulation combining three active substances, dinotefuran, pyriproxyfen and permethrin (DPP) (Vectra® 3D; Ceva Santé Animale SA, Libourne, France), was launched as an ectoparasiticide for dogs to treat and prevent flea and tick infestations and to repel sandflies, mosquitoes and stable flies (European Medicines Agency, 2013). The formulation also provides persistent insecticidal and repellent activity for 1 month against mosquitoes [*Stegomyia aegypti* (= *Aedes aegypti*)], stable flies [*Stomoxys calcitrans* (Diptera: Muscidae)] and bugs [*Triatoma infestans* (Hemiptera: Reduviidae)]. This ectoparasiticide combination had already been tested on dogs against *S. aegypti* mosquitoes and had exhibited high levels of anti-feeding and insecticidal efficacy (Franc *et al.*, 2012). However, no data on the effectiveness of DPP against *S. albopicta* were available.

The current experiment evaluated both the repellent and insecticidal effects of a dinotefuran–pyriproxyfen–permethrin spot-on formulation against *S. albopicta* mosquitoes on mice up to 4 weeks after the administration of treatment. The mouse model may be easier to set and is less expensive than the canine model. It is noteworthy that other animal species, such as chickens, pigeons, dogs and goats, have been used as models to evaluate the insecticidal and repellent efficacy of fipronil and imidacloprid against *T. infestans* (Gentile *et al.*, 2004; Carvajal *et al.*, 2014).

Materials and methods

Mice and ethics statement

Twenty-two healthy, adult (6-week-old), female Swiss CD1 mice (Charles River Laboratories, Écully, France) were used in this investigation. The mice were housed in groups of three to five individuals per cage (50 × 20 × 20 cm) at a temperature of 22 °C under an LD 12 : 12 h cycle. They were supplied with food pellets and water *ad libitum*. After administration of the test agent (DPP), mice were observed once per day for abnormal clinical signs. The experimental protocol and procedures were reviewed and approved by the Ethics Committee for Animal Experimentation at Aix-Marseille University (approval no. 2015040912442281). The mice were handled according to French legislation for the protection of animals used for scientific purposes (decree no. 2013–118; 1 February 2013, Paris) (Legifrance, 2013).

Treatment

Vectra® 3D contains three active ingredients, comprising dinotefuran [(2-methyl-1-nitro-3-(tetrahydrofuran-3-ylmethyl) guanidine)], pyriproxyfen [(4-phenoxyphenyl (RS)-2-(2-pyridiloxo) propyl ether)] and permethrin [(3-phenoxybenzyl (1RS)-*cis*, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate)] at 54.00 mg, 4.84 mg and 397.00 mg per mL, respectively. The formulation is an ectoparasiticide designed for use in dogs. The minimum recommended dose used to treat dogs [i.e. 6.4 mg/kg bodyweight (bwt) of dinotefuran, 0.6 mg/kg bwt of pyriproxyfen and 46.6 mg/kg bwt of permethrin] served to determine the dose to be administered to mice weighing a mean ± standard deviation (SD) of 20.16 ± 0.4 g. As DPP is a topically applied non-systemic drug, it was decided that doses expressed in mg/kg bwt for dogs should be converted to equivalent surface area doses in mice expressed as mg/m² (Table 1), as previously described by Freireich *et al.* (1966). The body surface area of an adult Swiss mouse was considered equal to 0.0066 m². The dose of DPP was estimated at 0.014 mL/mouse. The mouse's bristles were separated and the formulation was applied directly to the skin as a line-on treatment along the length of the spine using a micropipette. To ensure a uniform impregnation of the active ingredient across the entire body of the mouse, the treatment was applied 18 h before the first exposure to mosquitoes.

Source of S. albopicta

Stegomyia albopicta mosquitoes (Marseille strain) used in this study were laboratory-reared from 2012 at the present group's laboratory's insectarium (Aix-Marseille University, Marseille, France). Mosquitoes were kept in a climate-controlled chamber (Sanyo incubator MIR-254-PE; Sanyo Electric Co. Ltd, Tokyo, Japan) at a temperature of 28 °C and relative humidity (RH) of 70–90%. Adults were fed on a 10% sugar water solution. For reproduction purposes, the mosquitoes were fed with defibrinated human blood (according to agreement

Table 1. Dose estimation of Vectra® 3D administered in mice.

	MRD in dog, mg/kg bwt	Conversion factor	Dose in mouse, mg/kg bwt	km factor	Dose in mouse, mg/m ² BSA	Dose per mouse, mg*
Dinotefuran	6.4	6	38.4	3	115.2	0.76
Pyriproxyfen	0.6	6	3.6	3	10.8	0.071
Permethrin	46.6	6	279.6	3	838.8	5.53

*In Swiss mice: body weight = 0.02 kg; BSA = 0.0066 m².

Conversion factor and kilometre (km) factor are constants reported by Freireich *et al.* (1966).

BSA, body surface area; MRD, minimal recommended dose.

with the Etablissement Français du Sang) using a haemotek membrane feeding system (Discovery Workshops, Accrington, U.K.). Female *S. albopicta* aged 3–4 days were used in the present study. All mosquitoes were starved for 24 h before the experiment.

Mouse exposure to *S. albopicta*

After an acclimation period of 10 days, the mice ($n=22$) were randomly allocated into two equal groups consisting of an untreated control group and a DPP-treated group. The two groups were maintained in isolation, under similar conditions, for the duration of the study. Female mosquitoes were allocated to 22 cages at a mean \pm SD of 27 ± 2 mosquitoes per cage. Mosquitoes were observed for approximately 2 h before testing to ensure that damaged specimens were not included. All mice were exposed for 1 h to mosquito infestation on days 1, 7, 14, 21 and 28 post-treatment. Before exposure, each mouse was anaesthetized with an intraperitoneal injection of a combination of 90 mg/kg bwt ketamine (Imalgene® 500; Merial SA, Lyon, France) and 10 mg/kg bwt xylazine (Rompun® 2%; Bayer Santé Animale SA, Lyon, France), and was then introduced into a mosquito cage ($17 \times 17 \times 17$ cm). Mice were observed regularly during and after treatment and infestation to record adverse reactions and clinical side-effects. After the 1-h exposure period, the mosquitoes were categorized and counted as engorged/non-engorged and dead/live. All live mosquitoes were placed in separate incubators and fed on sugar water. Dead mosquitoes were counted after 24 h of observation. Moribund specimens and those that had lost legs were considered as dead.

Statistical analysis

The geometric mean numbers of engorged mosquitoes and live mosquitoes were calculated at each time-point. Levels of anti-feeding and insecticidal efficacy were calculated using Abbott's formula (Abbott, 1987) as:

$$\text{insecticidal efficacy (\%)} = 100 \times \frac{MC - MT}{MC}$$

where MC represents the mean [geometric mean (GM) or arithmetic mean (AM)] number of live mosquitoes in the control group, and MT represents the mean (GM or AM) number of live mosquitoes in the treated group, and

$$\text{anti-feeding efficacy (\%)} = 100 \times \frac{MC - MT}{MC}$$

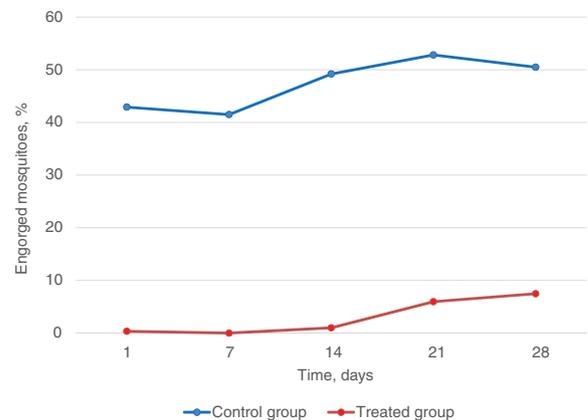


Fig. 1. Engorgement rates of *Stegomyia albopicta* in the control group (untreated mice) and treated group (mice treated with a combination of dinotefuran, pyriproxyfen and permethrin). [Colour figure can be viewed at wileyonlinelibrary.com].

where MC represents the mean (GM or AM) number of engorged mosquitoes in the control group, and MT represents the mean (GM or AM) number of engorged mosquitoes in the treated group.

Statistical analyses were performed using STATISTICA Version 6.1 (StatSoft Inc., Tulsa, OK, U.S.A.). At each time-point, differences between treated and untreated mice were compared using Student's *t*-test and the chi-squared test. *P*-values of ≤ 0.05 were considered to indicate differences of statistical significance.

Results

No adverse effects of treatment were observed in any of the treated mice. Female mosquitoes landed on the mouse, preferentially on hairless parts such as the muzzle, ears, paws and tail. In cages containing treated mice, mosquitoes were unable to take bloodmeals, with the exception of a few specimens ($< 1\%$), on days 0, 7 and 14. After day 14, blood-feeding behaviour increased and $> 5\%$ of mosquitoes fed on days 21 and 28. In the untreated control mice, mosquitoes showed conspicuous signs of engorgement at 4–6 min after contact with the mouse.

Anti-feeding efficacy

Throughout the study, the rate of engorgement in female mosquitoes ranged from 42.9 to 50.4% and from 0 to 7.3% in

Table 2. Percentage repellency of Vectra® 3D against *Stegomyia albopicta* in mice.

Exposure day	Number of fed mosquitoes, geometric mean \pm SD		Repellency efficacy, %
	Untreated group	Treated group	
1	11.81 \pm 2.83	0.09 \pm 0.30	99.23%*
7	11.09 \pm 1.70	0	100%*
14	13.90 \pm 2.60	0.27 \pm 0.64	98.03%*
21	15.36 \pm 3.50	1.63 \pm 1.02	89.34%*
28	14.18 \pm 3.66	2.00 \pm 1.00	87.35%*

*Significant difference between the treated and untreated groups ($P < 0.0001$).

SD, standard deviation.

the control group and treated group, respectively (Fig. 1). The GM number of fed mosquitoes in the untreated group ranged from 11.81 to 14.18. Levels of anti-feeding efficacy of DPP were 99.2, 100, 98.0, 89.3 and 87.4% at days 1, 7, 14, 21 and 28, respectively (Table 2).

Insecticidal efficacy

The GM numbers of live mosquitoes in the treated and control groups were calculated at 1 h and 24 h post-exposure on each day of the experiment (Table 3). The number of live mosquitoes remained higher ($P < 0.0001$) in the control group than in the treated group throughout the study. Levels of insecticidal efficacy in the treated group compared with the control group on days 1, 7, 14, 21 and 28, respectively, were 36.7, 28.9, 30.8, 23.1 and 11.9% at 1 h, and 68.4, 45.0, 43.3, 37.9 and 19.9% at 24 h (Table 3).

Discussion

The present study allowed for the quantification of blood-feeding rates of *S. albopicta* on Swiss mice, as well as the determination of the anti-feeding and insecticidal repellency effects of a topical treatment using a mouse model. As this model has not been reported previously in the literature, the first aim of the study was to confirm that *S. albopicta* mosquitoes blood feed on mice in order to indicate the validity of this approach.

In veterinary medicine, target species such as dogs are often involved in parasiticide testing without preliminary efficacy assessments in animal models (Tiawsirisup *et al.*, 2007; Machida *et al.*, 2008; Bonneau *et al.*, 2010; Franc *et al.*, 2012; Molina *et al.*, 2012; Dumont *et al.*, 2015a, 2015b; Varloud & Hodgkins, 2015). However, these models can provide preliminary and complementary information that may be useful in further testing on the targeted animal. In the present study, the mouse model was selected to explore the repellent and insecticidal efficacy of DPP prior to demonstration in the target species (dogs) because this model is fast and easy to perform. Reifensath & Rutledge (1983) described a dose–response method for testing *S. aegypti* repellents on mice. In a subsequent study conducted by Rutledge *et al.* (1994), the objective was the evaluation of the repellent effectiveness of eight commercial products against *S. aegypti* using a laboratory mouse model and volunteers. Findings showed that the results obtained with mice were representative of those obtained with human volunteers. Although representativeness can always be questioned, the information collected from the models is definitely useful in terms of optimizing experimental design in investigations in the target animal.

In the current study, the feeding rate of *S. albopicta* on control mice ranged between 41.4 and 52.8% and was slightly higher than that reported in a previous dog study (24.4–50.6%) using the same mosquito species (Fankhauser *et al.*, 2015). The high feeding rate showed that the population of *S. albopicta* in the experiment was robust (the mosquito population did not suffer any abnormality as a result of taking a bloodmeal from mice), which allowed the effect of the anti-feeding product to be validated. The host-seeking behaviour of female *S. albopicta* had been evaluated previously with reference to mice in a study that found the host-seeking activity of 6-day-old *S. albopicta* mosquitoes gradually increased to a maximum of 35.7% when they were given up to 30 min of exposure once per day for 6 days post-emergence (Fukumitsu *et al.*, 2012).

In the present study, the survival rate of mosquitoes in the control group was also satisfactory, with > 99.0 and $> 93.2\%$ of mosquitoes surviving until 1 h and 24 h, respectively, after the end of the exposure period. These data concord with the World Health Organization recommendation that mortality in the control group should not exceed 20% to validate the experiment (World Health Organization, 2006). Although the treatment was applied at the lowest recommended dose, the repellency of

Table 3. Insecticidal efficacy of Vectra® 3D against *Stegomyia albopicta* in mice at 1 h and 24 h post-exposure.

Exposure day	Number of live mosquitoes, geometric mean \pm SD				Insecticidal efficacy, %	
	1 h		24 h		1 h	24 h
	Untreated group	Treated group	Untreated group	Treated group		
1	27.44 \pm 0.8	17.44 \pm 3.8	26.52 \pm 1.1	8.37 \pm 1.2	36.65%*	68.41%*
7	26.72 \pm 0.4	19.01 \pm 1.8	25.53 \pm 2.0	14.04 \pm 5.1	28.85%*	44.96%*
14	27.99 \pm 0.7	19.35 \pm 3.0	26.33 \pm 1.3	14.92 \pm 2.8	30.84%*	43.30%*
21	28.19 \pm 0.4	22.30 \pm 2.6	27.19 \pm 0.8	16.89 \pm 3.8	23.07%*	37.89%*
28	28.08 \pm 0.5	24.75 \pm 2.8	26.60 \pm 1.2	21.31 \pm 2.6	11.85%*	19.90%*

*Significant difference between the treated and untreated groups ($P < 0.0001$).

SD, standard deviation.

DPP against *S. albopicta* was higher than that reported by Franc *et al.* (2012) using the same formulation against *S. aegypti* in dogs. However, repellency was close to that reported for other commercial products containing permethrin tested against *S. albopicta* in dogs (Fankhauser *et al.*, 2015). Permethrin is both an insecticide and a repellent. It acts against insects through contact (Brown & Hebert, 1997; Beugnet & Franc, 2012). The repellency of a single-active ingredient 65% permethrin spot-on formulation against *S. aegypti* remained at <89.9% during the first 3 weeks following administration and then decreased to 61.9% at 28 days after treatment (Meyer *et al.*, 2003). Indeed, additive effects were observed in numerous studies in which permethrin was administered in combination with other insecticides. In the same species of mosquito, a formulation containing 10% imidacloprid and 50% permethrin provided repellency that ranged from 84.9 to 94.1% until day 21 and then declined to 50.4% at 28 days after treatment (Tiawsirisup *et al.*, 2007). In a recent combination, 6.7% fipronil and 50.4% permethrin continued to have high repellency against *S. aegypti* (96.1%) and *Culex pipiens* (Diptera: Culicidae) (90.4%) for 28 days post-treatment. For *S. albopicta*, repellency in dogs ranged from 93.4 to 96.6% until day 21 and then decreased slightly to 86.9% on day 28 (Fankhauser *et al.*, 2015). The DPP formulation tested in the current study provided a good anti-feeding effect in mice, ranging from 89.3 to 100% to day 21 and remaining at 87.4% on day 28.

In this study, DPP exhibited a moderate mortality effect against *S. albopicta*. At 1 h after exposure, insecticidal efficacy ranged from 23.1 to 36.7% for 21 days and declined to 11.9% at day 28 post-treatment. Nevertheless, the GM number of live mosquitoes calculated at 1 h post-exposure remained significantly higher in the control group than in the treated group throughout the study. This confirms the insecticidal potential of DPP against *S. albopicta*. However, it should be noted that all the mosquitoes that fed on treated mice died within hours following the experiment, proving the insecticidal effect of DPP by contact. Additionally, insecticidal efficacy calculated 24 h after exposure ranged between 68.4 and 37.9% until day 21, and decreased to 19.9% on day 28. These levels are lower than those reported by Fankhauser *et al.* (2015), who tested fipronil and permethrin against *S. albopicta*. Their results show efficacy above 97.1% at 24 h post-exposure for at least 1 month after application of the product (Fankhauser *et al.*, 2015). This difference in insecticidal efficacy can be explained by the length of contact time between the mosquito and the treated animal. Both dinotefuran and fipronil act through contact on arthropods, and cause hyperexcitation (rapid, brief and inconsistent movements) that leads to death (Corbel *et al.*, 2004; Beugnet & Franc, 2012).

In this study, repellent and insecticidal efficacy against *S. albopicta* was demonstrated using a mouse model. In addition, the test agent has been proven to be effective against *S. aegypti*, *Phlebotomus perniciosus* (Diptera: Psychodidae), ticks [*Rhipicephalus* spp., *Ixodes* spp., *Dermacentor* spp., *Amblyomma* spp. (all: Ixodida: Ixodidae)] and fleas [*Ctenocephalides felis*, *Ctenocephalides canis* (Siphonaptera: Pulicidae)] in dogs (Coyne, 2009; Franc *et al.*, 2012; Lienard *et al.*, 2013; Varloud & Fourie, 2015; Varloud & Hodgkins, 2015). A recent study, in which DPP was tested against *T. infestans* using a rat model, showed that DPP has a powerful effect against *T. infestans* from

day 1 of administration that lasts for at least 3 weeks (Tahir *et al.*, 2017). Hence, the monthly use of DPP on dogs can be considered as a safe and effective measure against ectoparasites and associated vector-borne diseases.

Conclusions

The present study, based on the mouse model, demonstrates that the dinotefuran–pyriproxyfen–permethrin combination has reliable repellency and insecticidal efficacy against *S. albopicta*. In mice, this efficacy persisted for at least 1 month after treatment. As the tested dose was designed to be representative of the minimal recommended dose in dogs, these results can be extrapolated to dogs. Previous studies have reported that the results obtained in mouse models and in human volunteers were similar in terms of the repellent effectiveness of eight products against *S. aegypti*. (Rutledge *et al.*, 1994). Therefore, the present findings strongly suggest that DPP will show good efficacy against *S. albopicta* in dogs.

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