Deltamethrin and permethrin are used to prevent culex and sandflies from feeding on dogs (Killick-Kendrick et al., 1997; Xiong et al., 1994; Xiong et al., 1995). Products with these active ingredients have the claim “antifeeding effect on culex and sandflies” and also the claim “treatment and prevention of fleas and ticks infestation”. Two new formulations with new active ingredient (pyriprole alone, a phenylpyrazole insecticide and metaflumizone, a semicarbazone insecticide combined with an acaricide the amitraz) used in spot-on appeared on the market. The claims are similar “treatment and prevention of fleas and ticks infestation”. The formulation is applied on the skin (one point), then the active ingredient enters the lipid layer of the skin and spread rapidly across the all surface of the dog.

It appears interesting for the user to know in leishmaniosis area if these new formulations are able to protect dogs from sand flies biting.

MATERIALS AND METHODS

DOGS

Twelve adult male and female Beagle dogs, weighing between 8.5 and 12.1 kg were housed individually in stainless steel cages in an environmentally controlled room, fed a commercial dog food with water supplied ad libitum. All dogs were identified by ear tattoo. After two weeks acclimatization and before treatment, all were challenged with unfed adult female sandflies, ranked according to the number of sandflies biting them into four partner triplets of approximately equal sensitivity and randomly allocated to one of the three groups (treated with pyriprole spot-on, treated with metaflumizone-amitraz spot-on or left untreated) using a random ABC sequence.

Summary:

A controlled clinical trial was performed to assess the effectiveness of a pyriprole (125 mg/ml) and a metaflumizone (150 mg/ml) combined with amitraz (150 mg/ml) spot-on treatment (recommended dosage) in preventing adult female sandflies (Phlebotomus perniciosus) from feeding on dogs. Sandfly mortality was also assessed. Twelve beagle dogs were used in the study. Prior to treatment they were checked for their attractiveness to sandflies, ranked accordingly to generate partner triplets of equivalent sensitivity to sandflies: four control dogs, four treated with the pyriprole and four with the metaflumizone spot-on. The dogs were challenged with 50 unfed adult female sandflies (8-10 days old), in cages for one hour on Day 1 and Day 7. The sandflies were checked for blood feeding and mortality at one hour, 24 hours and 48 hours after exposure to the dogs. A very poor antifeeding effect (near 7 %) was seen on sandflies with the metaflumizone combined with amitraz and no antifeeding effect was seen with pyriprole. The sandfly mortality effect as a result of exposure to treated dogs was under 20 % for the two spot-on. The two formulations could not be proposed in a leishmaniosis prevention program.

KEY WORDS: metaflumizone, pyriprole, Phlebotomus, sandfly control, antifeeding, dog, spot-on.

Résumé : Pyriprole et association metaflumizone-amitraz : étude de l’activité anti-gorgement vis-à-vis de Phlebotomus perniciosus sur le chien traité par ces formulations spot-on

Cet essai avait pour but d’étudier l’efficacité de deux spot-on destinés au chien – pyriprole (125 mg/ml) et metaflumizone (150 mg/ml) associée à l’amitraz (150 mg/ml) – sur les phlébotomes (effet létal et effet antigorgement). 12 chiens ont été utilisés. Ils ont été répartis en trois lots de quatre en fonction de leur attractivité pour les femelles de phlébotomes. Un lot a été traité avec le spot-on au pyriprole, un lot avec la metaflumizone associée à de l’amitraz, le dernier lot étant le lot témoin non traité. Un et sept jours après le traitement, les chiens ont été soumis pendant une heure à des infestations par 50 femelles à jeun de Phlebotomus perniciosus âgées de 8-10 jours. L’état de gorgement a été observé une heure après, la mortalité notée 24 et 48 heures après chaque infestation. L’effet antigorgement est faible (7 %) pour la metaflumizone associée à l’amitraz et nulle pour le pyriprole. La mortalité entraînée par les deux produits est d’environ 20 %. Ces deux formulations ne présentent pas d’intérêt dans un programme de prévention de la leishmaniose.

MOTS CLÉS : métaflumizone, pyriprole, Phlebotomus, prévention phlébotome, antigorgement, chien, spot-on.
None of the dogs had received any antiparasitic treatment, which had ectoparasiticidal activity within two months of the start of acclimatization. Dogs were observed regularly throughout the study.

**Sandflies Maintenance and Supply**

*Phlebotomus perniciosus* (Diptera, Psychodidae), originally sourced from Lisbona (Dr Carlos Pires-Portugal), were cultured at ENVT using a 5-week egg to adult cycle. 50 adult females 8-10 days old were used to challenge each dog.

**Experimental Procedure**

The dogs were treated with the unit label dose of each formulation respecting the laboratories recommendations. For each product, the unit label dose of solution was applied topically by first parting the hair on the neck between the shoulders and applying the formulation directly on a single spot on the skin.

Dogs of the group A were untreated (Control group); dogs of group B were treated with a pyriprole spot-on (Prac-tic 137.5 mg® or Prac-tic 275 mg® depending of the weight of the dog, Novartis) at the dosage of 13.7-24.9 mg/kg; the dogs of group C were treated with a metaflumizone-amitraz spot-on (PromerisDuo 499.5 mg + 499.5 mg® Fort Dodge) at the dosage of 39-46 mg/kg of each active ingredient.

The sandfly challenge assessment cages (60 cm × 40 cm × 50 cm) were constructed from sandflies netting mounted on a wooden frame and placed in environmentally controlled rooms. Fifty unfed female sandflies were placed in each of these four hours before introducing the dogs, which were immobilised using ketamine (Imalgene® 9 mg/kg, Merial) and medetomidine (Domitor® 4 µg/kg, Pfizer) anaesthesia throughout the one hour exposure to the sand flies. Three partner pairs were challenged with sandflies at any one time and all challenge assessments were done in virtual darkness except for some borrowed light to enable the dogs to be observed throughout the challenge period.

At the end of the exposure period the dogs were checked carefully for any sandflies remaining on them, then removed from the cage and allowed to regain full consciousness. All the live female sandflies collected, observed and classified (engorged or unengorged) and placed in holding cages for observation 48 hours. The dead present in the assessment cage were counted and classified (engorged or unengorged). The sandflies were fed on sugar-water and checked for mortality at 24 and 48 hours after exposure to the dog.

**Data Analysis**

The data collected at each time point were: number of live and engorged sandflies, number of live and un-engorged sandflies, number of dead and engorged sandflies, number of dead and un-engorged sandflies. Effects were assessed on a group basis (total numbers per treatment group). The statistical unit was the individual dog. The mortality and the anti-feeding rates were determined for each treatment group at each occasion of exposure of the dogs to sandflies (at 24 hours and at 48 hours after exposure) as follows:

\[
\text{mortality rate} = \frac{\text{total number of dead sandflies}}{\text{total number of recovered sandflies}}
\]

\[
\text{anti-feeding rate} = \frac{\text{total number of unfed sandflies}}{\text{total number of recovered sandflies}}
\]

The mortality effect and the anti-feeding effect of the treatment was calculated for each time point of assessment by the following formula:

\[
\text{mortality effect} = \frac{(\text{mortality rate in treated dogs}) - (\text{mortality rate in untreated dogs})}{1 - (\text{mortality rate in untreated dogs})}
\]

\[
\text{anti-feeding effect} = \frac{(\text{anti-feeding rate in treated dogs}) - (\text{anti-feeding rate in untreated dogs})}{1 - (\text{anti-feeding rate in untreated dogs})}
\]

Statistical analyses: comparisons between groups were performed using the non parametric test Kruskal-Wallis (p < 0.05).

**Results**

The results (Fig. 1) show that the experimental infestations with *Phlebotomus perniciosus* were successful, with a low percentage of mortality (4 to 18 %) of the insects exposed on untreated dogs. Between 41 and 48 mosquitoes were steel alive 48 hours after each exposure to the control dogs.

The mortality effect 48 hours after exposure to the treated dogs was approximately 20 % on D1 and 10 % on D7 with pyriprole and 16-11 % with metaflumizone. (Table I and Fig. 1). The mean number of alive female

<table>
<thead>
<tr>
<th>Day infestation</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyriprole</td>
<td>Metaflumizone</td>
</tr>
<tr>
<td>D1</td>
<td>7.20</td>
<td>0.00</td>
</tr>
<tr>
<td>D10</td>
<td>2.10</td>
<td>3.60</td>
</tr>
</tbody>
</table>

Table I. – Sandfly mortality effect (%).
is statistically different between the pyriprole treated group and the control group \((p < 0.05)\) on days 1 and 7 and on day 7 for the metaflumizone group comparing to the control group. In the same conditions the percentage of engorged females in the control group was between 84 and 86 % and approximately 92 % in the pyriprole treated group and 82 % in the metaflumizone treated group. (Fig. 2). The observed percentages of feeding did not differ significantly between the two treated groups and the untreated group on day 1 and 7 \((p < 0.05)\). The metaflumizone anti-feeding effect of sandflies after one hour exposure to treated dogs were approximately 7 % on D1 and D7. No antifeeding effect was observed with the pyriprole. (Table I and Fig. 2).

### DISCUSSION AND CONCLUSION

The two new spot-on pyriprole (125 mg/ml) and metaflumizone (150 mg/ml) combined with amitraz (150 mg/ml) are very efficient against fleas and ticks (Barnett et al., 2007; Hellmann et al., 2007; Rugg et al., 2007; Schuele et al., 2007).

In our trial conducted in the good clinical practice, we don't observe a significant difference in the killing effect between the two formulations 16 to 20 % at day 1 and 9 to 11 % on day 7. Only with the metaflumizone we observe a no significant antifeeding effect (near 7 %)
against *Phlebotomus perniciosus* one and seven days after the treatment at the recommended dosage. Consequently the dogs are not protected from being bitten by sandflies. So the two formulations could not be proposed in a canine leishmaniosis prevention program.

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**PARASITE**

**ADDENDUM AUX INSTRUCTIONS AUX AUTEURS**

**Règles de taxinomie**

Dans la page de titre, les taxa des parasites et des hôtes seront suivis soit de l’autorité du taxon et de la date de création, soit de n. gen. et/ou n. sp. Les catégories supra-familiale et familiale suivront entre parenthèses. Dans le chapitre “Matériel et Méthodes”, la classification choisie pour les hôtes sera précisée. Les données biologiques (prévalence, charge parasitaire, coparasitisme...) seront données dans la section taxinomique ainsi que l’étymologie du nom du taxon.

**ADDENDUM TO INSTRUCTIONS TO AUTHORS**

**Taxonomic rules**

In the title page, the taxa of parasites and their hosts will be followed either by the authority of the taxon and the date of creation, or by n. gen. or/and n. sp. Suprafamilial and family categories will follow in brackets.

In the chapter "Material & Methods", the choice of the classification for the host will be specified. Biological data (prevalence, burden, coparasitism...) will be provided in the taxonomic section as well as the etymology of the name of the taxon.