

| AB7 INDUSTRIES VETERINAIRES | | |
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Evaluation of a repellent spot on for dog

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1. Presentation of the product

We report the result of tests conducted to evaluate efficacy of a repellent spot-on for dog. The formulation tested in this report is the same used in the commercial product “*Pipette Zero puces moyen Chiens*”. This spot-on is to treat and prevent parasite infestation on dog. Product should be applied on wet dog, let act for few minutes, and then washed with clean water. This is a geraniol based product against flea and ticks.

“*Pipette Zero puces moyen Chiens*” is the property of *Hery Laboratoires*. “*Pipette Zero puces moyen Chiens*” (Ref: FZPMCHIEN5) is manufactured by *AB7 industries vétérinaires* under the control of *Hery laboratoires*.

2. Methods of evaluation

2.1. Preliminary information

2.1.1. Identification of the sample

- We used a sample with the same formulation than “*Pipette Zero puces moyen Chiens*” for these tests
- The sample used in these test was collected from stock production of *AB7 industries vétérinaires* the 23th June 2009.
- The sample was product the 15th April 2009. Batch number : P25824
- Tests were made the 23th of June 2009 for fleas and the 16th of December 2009 for the ticks.
- Geraniol is the active substance of the product (1.03 %).
- PH=6.55, density: 0.86.

2.1.2. Tests with Fleas

For test the repellent efficiency of the spray, we used a four-arm airflow olfactometer (Vet et al. 1983). At our knowledge there is not a published method to evaluate the repellent effect on fleas without the use of experimental animals. This test allows us to test only the repellent effect of the product which is an important distinction in the Directive 98/8/EC concerning the placing of biocidal products on the market. The use of this four-arm airflow olfactometer is currently use for evaluated attraction or repellent effect of divers product on a large scale of insects (e.g. Parra et al. 2009, Junker & Bluthgen 2008, Faccoli et al. 2008). We preferred the use of a four-arm olfactometer instead of Y or T-tubes olfactometers which do not create odour field that can be easily entered, left and re-entered. Moreover there is usually turbulences in Y and T tubes that can result in the mixing of odour offered (Vet et al. 1983)

2.1.3. Tests with Ticks

For test the repellent efficiency of the shampoo on ticks we used the Moving Object Procedure proposed by Dautel et al. (1999) and modified by Dautel (2003) for Insect Services. The interest of this method is to increase the motivation of the tick to move while displaying host seeking behaviour. For test efficiency of spot on we changed the protocol of the experiment by replacing the warmed drum used by Dautel (1999) by the hand of an human volunteer. We did it because the spot-on should be applied directly on skin and should be active after absorption by the skin. The classical method of the Moving object procedure was recently use to evaluate the efficiency of ContraZeck® a spray against ticks (Swantes et al. 2008), and a permethrine based spot on (Dautel & Cranna2006).

2.2. Description of the method

2.2.1. Tests with fleas

The objective of this test was to show the repellency of a spot on. For this test we used adults (males and females) rabbit fleas *Spilopsyllus cuniculi* (Dale) provided by Bio Espace laboratory, Montpellier (France). The fleas were maintained in glass vials in darkness at 4°C. Fleas were acclimated to room temperature for about 30 minutes before testing.

In the olfactometer 4 odour fields were created in the chamber by sucking air out through a hole in the centre of the floor. Each arm of the olfactometer was connected to a set of two 70 ml glass vials. The odour source was provided by the first vials, and the outer vials contained distilled water over which the incoming air was passed to create a high humidity. In two areas the first vials contained about 10 ml of Spot-on. In the two other areas the first vials were empty (controls).

Every minute we recorded the number of fleas in each area during 20 minutes. We use 45 fleas during this test. The fleas were tested in two groups of 8 to 16. Conditions of experiment were: light, 20°C, and humidity about 50%.

2.2.2. Tests with ticks

For this test we used spot on in its commercial form. We used free-disease nymphs of *Ixodes ricinus* L. both male and female. Ticks were provided by Insect Services, Berlin (Germany) and maintained in the laboratory less than two months before experiments in glass vial at a relative humidity about 90%, 21°C and natural photoperiod. The objective of this method was to attract ticks with a potential host and allows the tick to display their natural behaviour of clinging to passing host in the laboratory.

The human volunteer (A non smoking 29 years old man) placed a finger in face of a horizontally positioned glass rod at a distance of 1 to 2 mm. Ticks attracted to the warm and movement approached the finger on a glass rod. Then the tick was able to cling to the skin and transfer to the finger's volunteer. To test for repellency of the Spot on we applied 136mg of Spot-on on the hand of the volunteer. We waited for 10 minutes until all the spot-on were absorbed by skin, and the hand was dry. Then we recorded whether or not tick approaches and transfers to finger and if remains on it, walk away or drop off. The duration of each of these steps were also recorded. Time for walk to the top of the rod, time to attached, and time that the tick remained on the finger was monitored for a maximum of 2 minutes. There was 14 repetitions for the two groups (shampoo and control). Conditions of experiment were: light, 20°C, and humidity about 50%. There was 13 repetitions for Control and Spot On. Conditions of experiment were: light, 20°C, and humidity about 50%.

3. Results

3.1. Tests with Fleas

During 20 minutes, fleas were observed 284 times in odour field contaminated with spot-on, and 459 times in control area (fig 1). Therefore we found a significant repellency efficiency of the spot on ($\chi^2= 41.218$, d.f. = 1, $P<0.001$)

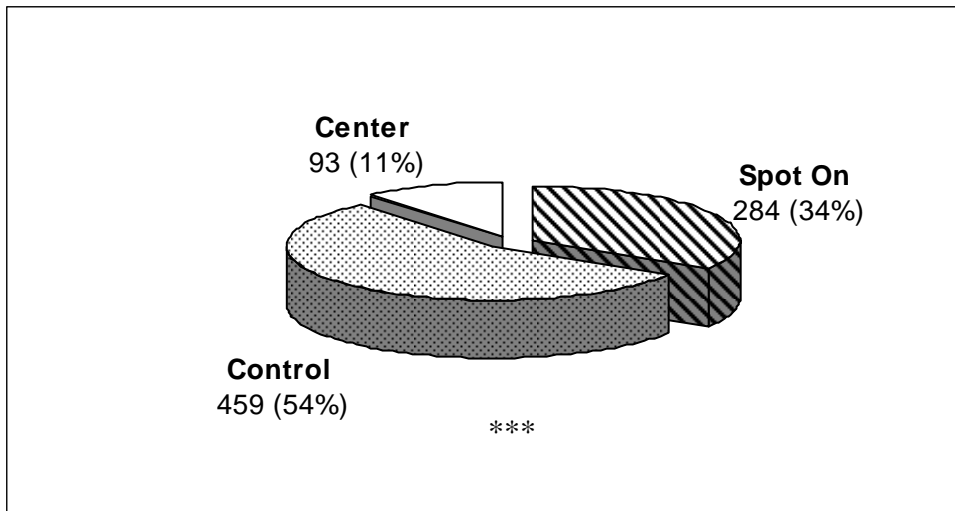


Figure 23: Number and percent of observation of fleas in each area. Stars show high significant differences ($\alpha < 0.001$).

3.2. Tests with Ticks

Ticks of the two treatment walked on the top of the road with the same rate ($\chi^2=2.16$, d.f.=1, $P=0.14$; fig 2). However, ticks clung on skin with lower proportion than control ($\chi^2=2.88$, d.f.=1, $P < 0.1$; fig 2). We also find that significant more ticks stay on skin more than two minutes ($\chi^2=2.88$, d.f.=1, $P < 0.1$, fig 2).

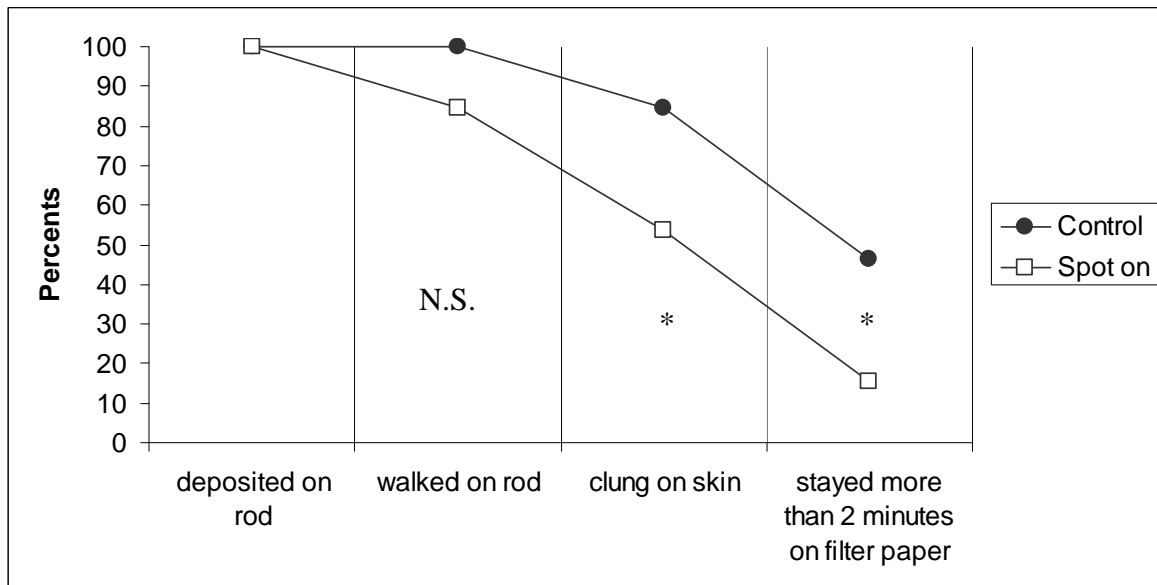


Fig 2: behaviour of ticks at each step of their foraging activity. Star show significant difference ($\alpha < 0.1$). N.S: Non significant.

Ticks took significantly more time to approach the top of the road in presence of spot-on (Kruskal-Wallis test, $\chi^2=5.35$, d.f= 1, $P < 0.05$; fig 3), but we found no differences for the time to catch the skin of the volunteer (Kruskal-Wallis test, $\chi^2=1.95$, d.f= 1, $P=0.163$; fig 3), and for the resident time on the finger (Kruskal-Wallis test, $\chi^2= 0.26$, d.f= 1, $P=0.61$; fig 3).

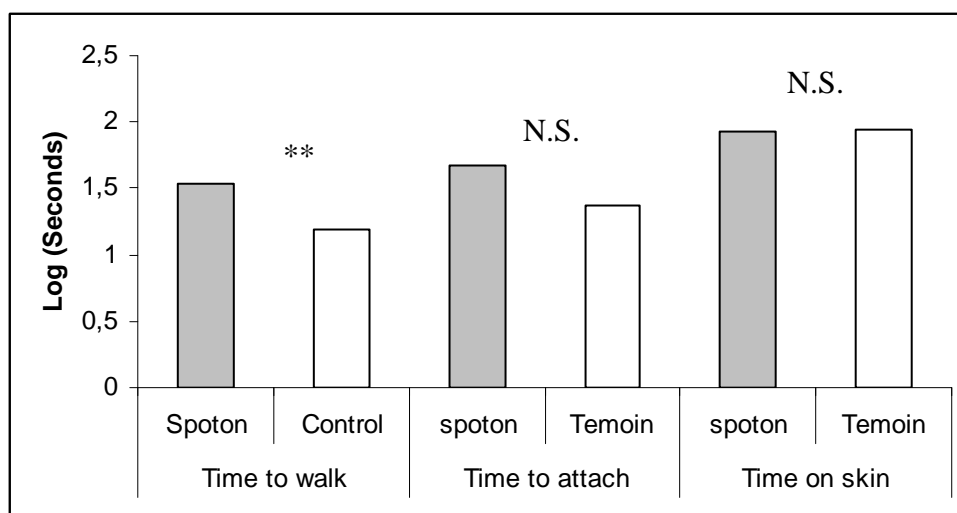


Fig 3: Time recorded for the three steps of the catching behaviour of the ticks. Stars show significant difference ($\alpha < 0.05$)

4. Conclusion

Based on the tests that we performed we can conclude to the high efficiency of “*Pipette Zero puces Chiens moyen*” of *Hery laboratoires*” against fleas and ticks.

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