

AB7 INDUSTRIES VETERINAIRES		
<i>BP 9 Laboratory of Entomology 31 450 Deyme, FRANCE.</i>	Contacts: x.martini@ab7-industries.fr Manager: Jean-Pierre Lautier: jp.lautier@ab7-industries.fr	13 th December 2009 5 pages

Evaluation of a repellent spray for dog

1.	Presentation of the product.....	2
2.	Methods of evaluation	2
2.1.	Preliminary information	2
2.2.	Description of the method	4
3.	Results	4
3.1.	Tests with fleas	4
3.2.	Tests with ticks	5
4.	Conclusion.....	6
5.	References	6



1. Presentation of the product

We report the result of tests conducted to evaluate efficacy of a repellent spray for dog. The formulation tested in this report is the same use in the commercial product “*Spray Zero puces Chiens*”. The spray is to treat and prevent parasite infestation on dog. Product should be applied on dog, and simply let dry. This is a geraniol based product against flea and ticks. “*Spray Zero puces Chiens*” is the property of *Hery Laboratoires*. “*Spray Zero puces Chiens*” (Ref: FZPSPCHI250) 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 “*Spray Zero puces Chiens*” of *Hery Laboratoires* in these tests.
- The sample used in these test was collected from production of *AB7 industries vétérinaires* the 15th June 2009.
- The sample was product the 29th April 2009. Batch number : P25824
- Tests started 15th June 2009 and finished the 18th June 2009.
- Geraniol is the active substance of the product (0.41 %).
- PH=6, density : 1.

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 spray on ticks we used the Moving Object Procedure founded by Dautel et al. (1999) and changed 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. In absence of potential host for test (human volunteers or experimental animals) it is the test that is the most close to real situation. This methods 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 the spray. 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.

Every minute we recorded the number of fleas in each area during 20 minutes. We use 21 fleas during this test. The fleas were tested in two groups of 4 to 10. Conditions of experiment were: light, 20°C, and humidity about 50%.

2.2.2. Tests with ticks

For this test we used spray in its commercial form. We used nymphs of *Ixodes ricinus* L. both male and female. Ticks were collected in the field near Tours, France and maintained in the laboratory less than one month 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 warm and movement and allows the tick to display their natural behaviour of clinging to passing host in the laboratory.

A glass balloon of water was heated to 38°C- 42°C. The temperature of the balloon was controlled with a thermometer. On the balloon there was a 50cm² filter paper serving as attachment site for the ticks. The balloon was gently moved during the experiment to simulate movement. Ticks attracted to the warm approached the balloon on an horizontally positioned glass rod that end directly in front of the filter paper at a distance of 1-2 mm. Then the tick was able to cling to the surface and transfer to the balloon. To test for repellency we applied 625 mg of the spray solution. For the control we used filter paper watered with 625mg of tap water. Then we recorded whether or not tick approaches and transfers to the balloon and if remains on the paper 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 attachment site was monitored for a maximum of 2 minutes. There was 20 repetitions for the two groups (spray and control). Conditions of experiment were: light, 20°C, and humidity about 50%.

3. Results

3.1. Tests with fleas

Fleas were recorded 114 times in area contaminated with spray odour, which is significantly lower than the number of times where fleas were noted in the control area ($\chi^2= 35.26$, d.f.=1, $P<0.001$, figure 1). Therefore we can conclude to the high efficacy of the product.

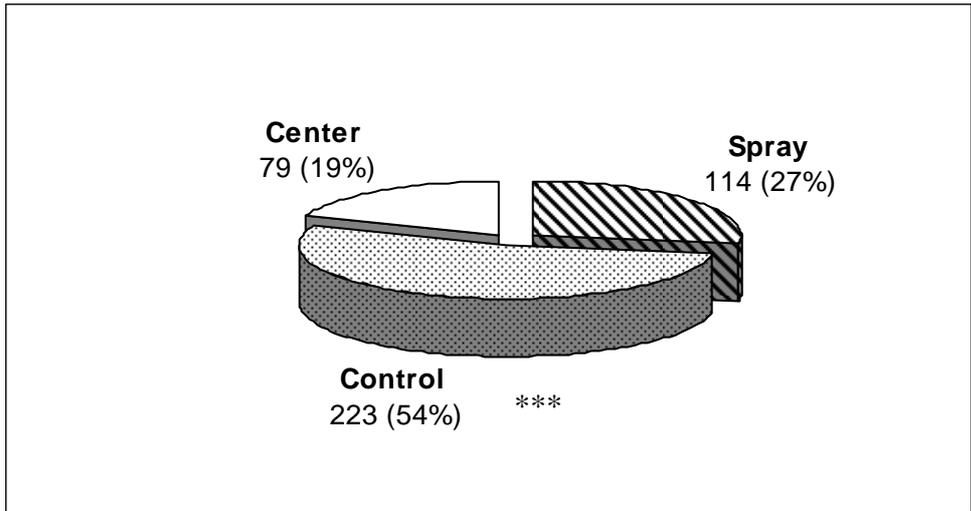


Figure 13: **Number and percent of observation of fleas in each area.** Stars show high significant differences ($\alpha < 0.001$).

3.2. Tests with ticks

There significantly less ticks that walk on the tip on the road with the spray treatment ($\chi^2=4.44, P=0.035$; Fig 1). Only 45% of ticks caught the filter paper with spray, which is significant too ($\chi^2=11.9, P<0.001$). Finally no ticks stay more than 2 minutes on the attachment site, which prove the high efficiency of the product ($\chi^2=36.2, P<0.001$, Fig 1).

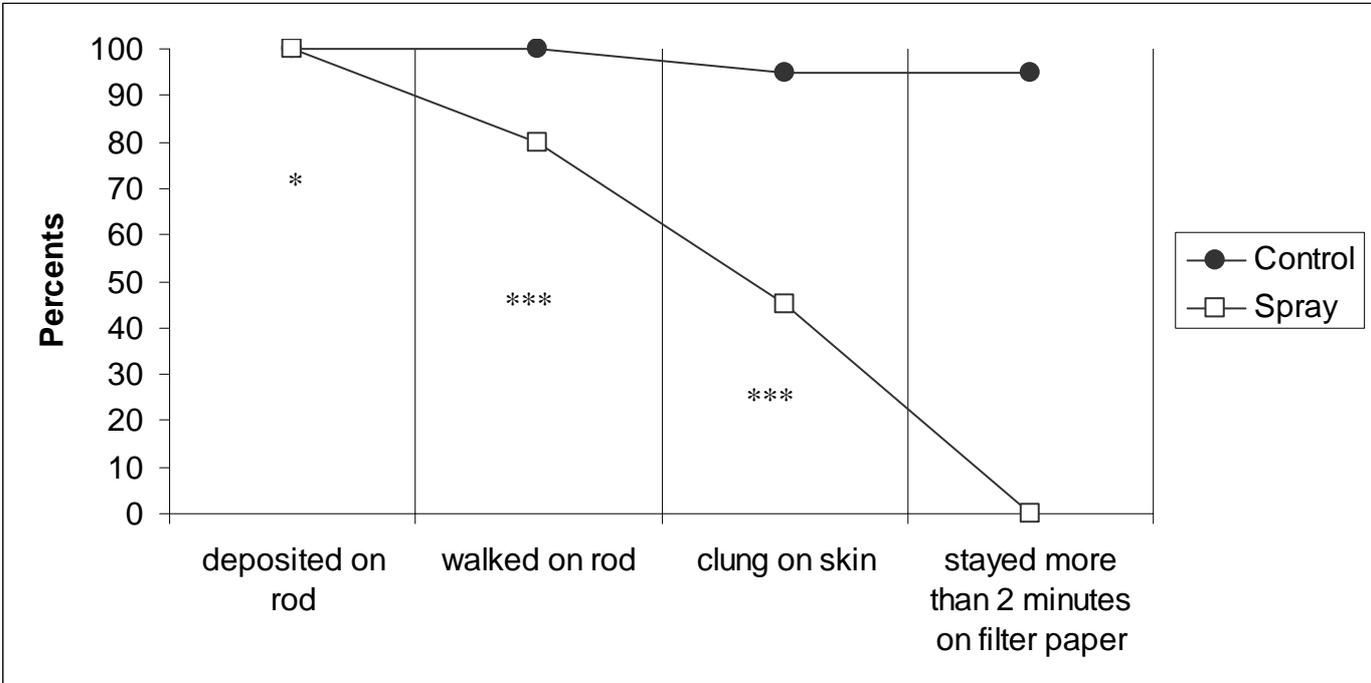


Figure 2: **behaviour of ticks at each step of their foraging activity.** Stars show significant differences (*: $\alpha < 0.05$; ***: $\alpha < 0.001$).

4. Conclusion

Based on the tests that we performed, we conclude that “*Spray Zero puces Chiens*” of *Hery laboratoires* has a strong repellent efficiency on fleas and ticks. On a comparable test it shows the same effect than the spray ContraZeck® used against ticks (Schwantes *et al.* 2008).

5. References

- Dautel, H. & Cranna, R. (2006) Assessment of repellency and mortality of a imidacloprid plus permethrin spot-on solution against *Ixodes holocyclus* using a moving object bioassay. *Australian Veterinary Practitioner*, **36**, 138-+.
- Dautel, H. (2003) Test systems for tick repellents. 7th *International Potsdam Symposium on Tick-Borne Diseases (IPS VII)*, pp. 182-188. Berlin, GERMANY.
- Dautel, H., Kahl, O., Siems, K., Oppenrieder, M., Muller-Kuhrt, L. & Hilker, M. (1999) A novel test system for detection of tick repellents. *Entomologia Experimentalis Et Applicata*, **91**, 431-441.
- Junker, R. R. & Bluthgen, N. (2008) Floral scents repel potentially nectar-thieving ants. *Evolutionary Ecology Research*, **10**, 295-308.
- Parra, L., Mutis, A., Ceballos, R., Lizama, M., Pardo, F., Perich, F. & Quiroz, A. (2009) Volatiles Released From *Vaccinium corymbosum* Were Attractive to *Aegorhinus superciliosus* (Coleoptera: Curculionidae) in an Olfactometric Bioassay. *Environmental Entomology*, **38**, 781-789.
- Schwantes, U., Dautel, H. & Jung, G. (2008) Prevention of infectious tick-borne diseases in humans: Comparative studies of the repellency of different dodecanoic acid-formulations against *Ixodes ricinus* ticks (Acari: Ixodidae). *Parasites & Vectors*, **1**.
- Vet, L.E.M; Van Lanteren J.C.V; Heymans, M; Meelis, E. (1983) An airflow olfactometer for measuring olfactory response. *Physiological Entomology*. 8:97-106.
- Faccoli, M., Anfora, G. & Tasin, M. (2008) Responses of the Mediterranean pine shoot beetle *Tomicus destruens* (Wollaston) to pine shoot and bark volatiles. *Journal of Chemical Ecology*, **34**, 1162-1169.