Efficacy of the Compound Preparation Imidacloprid 10% (w/v) / Permethrin 50% (w/v) Spot-on against Ticks (I. ricinus, R. sanguineus) and Fleas (C. felis) on Dogs

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STUDY OBJECTIVES AND RATIONALE

These studies were designed to confirm the acaricidal efficacy of the compound preparation Imidacloprid 10% (w/v) / Permethrin 50% (w/v) spot-on. Dogs, artificially infested with Brown Dog Ticks (Rhipicephalus sanguineus) and Castor Bean Ticks (Ixodes ricinus), and, in another study set infested with Cat Fleas (Ctenocephalides felis), were used as test system. The acaricidal efficacy against the two tick species and the flea species following a single topical treatment was assessed by repeated tick and flea infestations and subsequent tick and flea counts over a period of five weeks. These studies were conducted in accordance with the Principles of the VICH Guideline on Good Clinical Practices (GCPV) and the recommendations of the Guidelines for the Testing and Evaluation of the Efficacy of Antiparasitic Substances for the Treatment and Prevention of Tick and Flea Infestation in Dog and Cats (CVMP).

The test facility was inspected and certified as working in compliance with the Principles of GCPV. All procedures concerning animal husbandry and maintenance, hygienic measurements, randomisation, scalings, handling of test substances, retain samples, archiving and quality assurance are written down in current SOPs.

STUDY DESIGN

This blinded, negative controlled clinical laboratory GCP-study was designed with two parallel groups for each study set with tick infestation and flea infestation. After passing the study inclusion examination for each set 20 dogs were randomly allocated to two study groups of 10 animals per group. Group 1 of each set was treated with the investigational product in a dosage of 0.1 ml/kg bodyweight, the other group remained untreated. Dogs of both sets were reintroduced weekly with 50 adult Rhipicephalus and 50 adult Ixodes ticks (sex ratio: 1:1) or 100 adult Ctenocephalides felis fleas, respectively, for a period of five weeks. Efficacy against two tick species (I. ricinus/R. sanguineus) was tested 48 hours after regularly reinfestation on a weekly basis. (Fig. 1) Efficacy against adult fleas (C. felis) was tested 24 hours after treatment or regularly reinfestation on a weekly basis. (Fig. 2) Efficacy against juvenile fleas (laurae of C. felis) was tested with a blanket incubation test on a weekly basis. (Fig. 3) Tolerance was tested as second criterion also on a weekly basis.

MATERIAL AND METHODS

Ticks
1. Brown Dog Ticks (Rhipicephalus sanguineus) originated partly of the laboratory strain in Monheim, reared on rabbits and
partly of the laboratory strain of EL Labs Soquville in California/USA (ratio 50:50 Monheim: USA strain). The ticks for infestation were unengorged adult males and females (ratio 1:1) that have moulted at least 14 days before to the adult stage.

2. Castor Bean Ticks (*Ixodes ricinus*) originated from the Charité, Berlin. The ticks for infestation were unengorged males and females (ratio 1:1) in which the transmission of the spermatophore had already occurred.

**Fleas**

Cat fleas (*Ctenocephalides felis felis*) of the laboratory strain in Hanover, reared on cats, were used as test parasites. The adult unfed fleas were held in polyvinylic vials at ≈27°C/≈80% relative humidity until they were used for infestation (maximum for 12 days).

Viable flea eggs from the routine breeding of this flea strain were used as test parasites for the blanket larvicidal test. The flea eggs were at an age of max. 48 hours.

**Tick Infestation procedure**

After sedation dogs of study set 1 were placed in individual transport boxes. The ticks were released onto the back of the dogs and were allowed to disperse and move into the hair without disturbance. Dogs were released from the transport boxes after approx. 30 minutes.

**Flea Infestation procedure**

All dogs of study set 2 were artificially infested with about 100 unfed cat fleas by pouring the fleas together with cocoon material out of the vials onto the dog’s coat.

**Tick Counting Procedures**

The following examination procedure was followed: Protective clothing and disposable hand gloves were worn during the clinical examinations; gloves were changed between each dog. The dog was placed on a single-use paper pad covered table and was identified by ear tattooing.

All ticks were counted. Total body surface of the dog was examined by thumb counting, parting the hair with the fingers and removing the ticks with a forceps (excluding day 0 counts where the ticks were left in situ). The following regions were examined: head, ears, lateral areas, dorsal strip from shoulder blades to base of tail, tail and anal area, fore legs and shoulders, hind legs abdominal area from chest to inside hind legs, neck (application site). Dogs were combed until ticks were no longer found but for a minimum of 5 minutes. Live fleas were collected, removed and counted. Total counts of fleas were recorded.

**Blanket Incubation Test**

For assessment of the larvicidal properties of the test formulation in the surroundings of the dogs during the weeks after treatment, the dogs were placed on blankets for twelve hours a week divided into four contact intervals of 3 hours each. Blankets were exchanged weekly. One circular sample was cut from the middle part of each blanket after each study week, placed into individually marked plastic dishes and frozen at about –18°C for 24 hours to kill possible living fleas, larvae or eggs on the samples. For the incubation test approximately 50 flea eggs (originated from the same flea strain which was used for the adulticidal tests) were placed together with flea rearing medium on each fleece sample and were incubated at 27°C and 80% rel. hum. for four weeks. The number of the developing fleas was counted on day 28 after start of incubation.

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**Figure 1** Cat fleas (above); Castor bean ticks in all stages (below)
RESULTS

Efficacy was calculated comparing the tick counts in the treated group to the tick counts in the untreated group based on the geometric means as recommended in the guidelines.

The product's curative efficacy against *Rhipicephalus sanguineus* was 74.0% (day 2); the preventive efficacy was 94.0% (day 9), 97.6% (day 16), 92.0% (day 23), 95.9% (day 30) and 91.5% (day 37) (Tab. 1).

The product's curative efficacy against *Ixodes ricinus* was 67.0% (day 2); the preventive efficacy was 100.0% (day 9), 100.0% (day 16), 99.5% (day 23), 98.7% (day 30) and 91.6% (day 37) (Tab. 2).

The curative efficacy of the treatment against fleas was 99.4% (d+1); the preventive efficacy was 99.8% (day 8), 99.9% (day 15), 98.8% (day 22), 95.7% (day 29) and 90.4% (day 36) (Tab. 3).

The larvicidal efficacy on the blankets after 12 hours dog contact was 99.2% (d+3); 98.2% (day 10), 98.5% (day 18), 85.1% (day 24) and 50.2% (day 31) (Tab. 4).

The general and dermal tolerance of the product was very well and no adverse reactions were observed in any of the treated dogs during the study.

CONCLUSIONS

1) Efficacy against ticks

The investigational veterinary product had a curative efficacy against ticks of 74.9% in case of *Rhipicephalus sanguineus* and 67.0% in case of *Ixodes ricinus*. The preventive efficacy was clearly above 90% for a period of five weeks for both tick species. Preventive efficacy values were between 97.6% and 91.5% in case of *Rhipicephalus sanguineus* and 100% and 91.6% in case of *Ixodes ricinus*. Therefore the product proved to be effective against these tick species for a period of five weeks.

2) Efficacy against fleas

**Adulticidal Flea Efficacy**

The investigational veterinary product had an adulticidal curative efficacy against fleas on animals of 99.4% within 24 hours after treatment. The preventive efficacy was above 95% for a period of four weeks. Preventive efficacy values were between 99.9% and 95.8%. Therefore the product proved to be effective against fleas for a period of four weeks.

**Larvicidal Flea Efficacy**

The investigational veterinary product had a remarkable larvicidal efficacy against fleas on blankets that had been in contact for 12 hours to a treated animal. The efficacy values that were achieved were above 85% for a period of four weeks and ranged between 99.2% and 85.1%. Therefore the product proved to have a high protective effect against larval development in the animal’s direct surroundings throughout.

3) Tolerance

Imidacloprid 10% / Permethrin 50% spot-on was well tolerated by all ten dogs of the investigational veterinary product group concerning the general and dermal tolerance.