# Effectiveness of Two Topical Treatments With a Combination Fipronil/Amitraz/(S)-methoprene Against Natural Infestations of Mites (*Sarcoptes scabiei var. canis*) on Dogs.

Soila Gaxiola[1]

Joel Gaxiola[1]

Ascencion Perez[1]

Stephen Yoon[2]

Jennifer Irwin[2]

Lenaig Halos[2]

Frederic Beugnet[2]

Matthias Pollmeier[2]

Roberto Alva[2]

[1] *University of Sinaloa, School of Veterinary Medicine, Carretera Internacional, Salida Sur, Km. 3.5 Oriente, Culiacan, Sinaloa, Mexico.*

[2] *Merial, 3239 Satellite Blvd, Duluth, GA 30096-4640, USA*

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## ABSTRACT

The acaricidal efficacy of the novel topical combination of fipronil/amitraz/(S)-methoprene (CERTIFECT®) was evaluated against *Sarcoptes scabiei var canis* when administrated as topical solution to dogs naturally infested with the mites. A total of 24 mixed-breed dogs of both sexes (10 males and 14 females), aged from 0.3 to 5 years and weighing 3.2 to 20.2 kg, were studied in this single-center, randomized, blinded, and controlled trial. Dogs, naturally infested with *Sarcoptes scabiei var canis* as confirmed by skin scrapings collected prior to allocation, were randomly divided into two equal groups. Dogs in Group 1 were not treated. Dogs in Group 2 were treated on Days 0 and 28. At Days 0 (pretreatment), 7, 14, 21, 28 (before second treatment), 41, and 56, four skin scrapings of similar size were taken from different sites with lesions consistent with sarcoptic mange. Lesion scores were assigned weekly at Day 0 (pretreatment), 7, 14, 21, 28 (before second treatment), 35, 42, and 56, and silhouettes identifying the location of the present lesions were completed. The results showed that dogs treated with CERTIFECT® had significantly ($p<0.05$)

lower mite counts than untreated control animals at Days 14, 21, 41, and 56 at a percent reduction of 100%, 99.3%, 100%, and 100% respectively. In addition, dogs treated with CERTIFECT® had significantly ($p<0.05$) lower lesion scores than untreated control animals on Days 28, 35, 42, 49, and 56.

## INTRODUCTION

Canine sarcoptic mange is a highly contagious, intensely pruritic parasitic skin disease caused by infestation with the epidermal mite *Sarcoptes scabiei var canis*. The disease commonly affects domestic dogs with a worldwide distribution (Curtis, 1996). Over half of the dogs, and up to 50% of human companions, can develop skin lesions after having contact with infested dogs (Folz, 1984; Griffin, 1993; Ihrke, 1994). The mite is transmissible to humans (seen in up to 50% of cases), but because the mites cannot breed in human skin, humans are a dead-end host (Guaguère and Beugnet, 2008). Additionally, *S. scabiei var canis* has been isolated from species other than domestic and wild canids and has been established experimentally on several mammal species. This lack of host specificity has therapeutic and public health implications. In theory, all mammals in contact with an infested dog should be treated simultaneously to limit the opportunities for cross- and re-infestation (Curtis, 2004).

There are various topical therapies (lime sulfur, amitraz, organophosphates) and systemic therapies (milbemycin oxime, moxidectin, selamectin) currently used in the treatment of *S. scabiei* in dogs:

- acaricide amitraz dips (Curtis, 2004)
- selamectin as topical treatment (Shanks et al., 2000)
- moxidectin in topical combination with imidacloprid (Krieger et al., 2005)
- the combination of metaflumizone with the amitraz in a spot-on formulation (Fourie et al., 2007).

All were reported as being generally effective treatments for sarcoptic mange in dogs; nevertheless, some have limitations in their use and some are associated with adverse reactions (Folz, 1994; Griffin, 1993; Plumb, 1995; Curtis, 1996; Wendelberger and Wagner; 1998; Bordeau et Hubert, 2000; Curtis, 2004).

CERTIFECT® is a novel spot-on formulation which combines fipronil, amitraz and (S)-methoprene. The combination of fipronil and (S)-methoprene (FRONTLINE® Plus/Combo) formulated for topical application to dogs is known to provide a broad spectrum of activity against insects and acarids (Pollmeier et al, 2001; Curtis, 2004; Mc-Call et al, 2004; Tielemans et al, 2010), but it has been shown recently that the addition of amitraz to this combination potentiates significantly the acaricidal effects of fipronil (Pfister et al., 2011; Prullage et al, 2011.). The purpose of the present study was to confirm the efficacy of CERTIFECT® against natural infection with *Sarcoptes scabiei var canis*, when administered to dogs.

## MATERIALS AND METHODS

### Study design

This study was a randomized, blinded, controlled efficacy trial of a combination of fipronil/(S)-methoprene and amitraz against natural infections of *Sarcoptes scabiei var canis*. The experimental study unit was the individual dog, identified by name, and naturally infested with *S. scabiei var canis* as confirmed by skin scrapings collected prior to allocation to the study. The morphologic characteristics of the agent were determined according to the key published by Baker (1999).

In total, 24 mixed breed dogs of mixed sex (10 males and 14 females), aged from 0.3 to 5 years, and weighing 3.2 to 20.2 kg, were allocated to this study. The naturally-infested animals were obtained from private owners who consented to the animal's participation in the study.

The 24 *Sarcoptes*-infested dogs were divided randomly into two equal groups. Dogs in Group 1 were not treated. Dogs in Group 2 were treated on Days 0 and 28 with

the CERTIFECT® formulation of Fipronil/(S)-methoprene and amitraz concurrently administrated at a combined dose of 1.07 mL, 2.14 mL or 4.28 mL of total volume

| Body weight | Fipronil/ (S)-methoprene | Amitraz | Total volume |
|---|---|---|---|
| (kg) | (mL) | (mL) | (mL) |
| Up to 10.0 | 0.67 | 0.40 | 1.07 |
| 10.1-20.0 | 1.34 | 0.80 | 2.14 |
| 20.1-40.0 | 2.68 | 1.60 | 4.28 |

depending on the dogs body weight:

The minimum doses delivered were 6.7 mg/kg of fipronil, 6.03 mg/kg of (S)-methoprene, and 8 mg/kg of amitraz. For the treatment administration, the total volume was applied directly onto the skin on two separate spots placed on the midline of the neck, between the base of the skull and the shoulder blades.

No adverse events were observed during observations conducted approximately hourly for 4 hours immediately after the CERTIFECT® treatment administration.

In order to perform the mite counts and control the effectiveness of the topical treatments against the mites, four skin scrapings of similar size were taken from different sites with lesions consistent with sarcoptic mange on Days 0, 7, 14, 21, 28, 41, and 56. In the absence of skin lesions, scrapings were obtained from previously active sites. The collections on Days 0 and 28 were performed before treatments were applied to Group 2. Also lesion scores were assigned and their locations (if present) were identified on the Lesion Score form on Days 0, 7, 14, 21, 28, 35, 42, 49, and 56. The Lesions Scores on Days 0 and 28 were evaluated before treatments were applied to Group 2.

**Specification of Study Variables**

*Mite Counts*

Skin scrapings were collected and performed as follow: For each sample, an area of one square centimeter in size was scraped with a curette until blood began to appear. To ensure the attachment of the epidermal scrapings to the curette, one to two drops of mineral oil were placed on the curette prior scraping. The debris was removed from the collected sample with a 25-gauge needle prior to placing the scraping in a Petri dish containing 2 mL of mineral oil (one Petri dish per dog per day of analysis). Between each scraping, the curette was wiped with a paper towel and thoroughly scrubbed with alcohol, then wiped again.

The number of mites (live and dead) in the entire sample was counted using a light microscope.

**Lesion Score**

Sarcoptic mange lesion scores were assigned and their locations (if present) were noted on an animal silhouette. To maintain consistency, the same person evaluated each dog at each time point. Evaluation of individual dogs was conducted using a five-point scoring system:

0 no skin lesions

1 areas with papules and/or small areas with alopecia, $\leq 4$ areas over the body

2 areas with papules and/or small areas with alopecia, $> 4$ areas over the body

3 more than half of the body with papule and/or alopecia, crusts present

4 chronic infestations with crusts covering more than half of the body surface, skin reactions including alopecia or erythema.

**Data Analysis**

*Mite Counts*

The counts of live mites from the skin scrapings were summed for each animal for calculation of geometric means for each group at each time point. The total live mite counts were transformed to the natural logarithm of counts (count +1) for calculation of geometric means for each group at each time point. Arithmetic means also were calculated. Percent reduction from the control group mean was calculated for the treated group at every post-treatment time point using the formula $[(C-T)/C] \times 100$, where C is the geometric mean for the control group (Group 1) and T

is the geometric mean for the treated group (Group 2). The treated group was compared to the control group using Friedman's test.

**Lesion Score**

Lesion scores were summarized for each group and by time point and were analyzed by Cochran-Mantel-Haenszel test. All testing was two-sided at the significance level $\alpha = 0.05$. Supplemental descriptive statistics (standard deviation, minimum, and maximum) also were calculated. Analyses were performed using procedures of SAS® version 9.1.

**Animal Management**

As animals were obtained from private owners, an informed consent and agreement form was completed and signed by each owner before any study-related activities were performed. The animals were managed similarly and with due regard for their well-being. They were handled in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals and requirements of the local IACUC. All animals were housed in the same manner, individually in pens under controlled environmental conditions. Food and water were delivered ad libitum. On Day 0, all dogs were determined to be suitable for inclusion in the study on the basis both of physical examinations and of mite counts confirming infestation with *S. scabiei var canis*. Dogs treated with ectoparasiticides (either topical or systemic) within 3 months of the start of the study were excluded. Dogs were observed daily for any health changes from Day -7 until the end of the trial.

## RESULTS AND DISCUSSION

The mite counts and the lesion score during the 56 days of trial are summarized in the Table 1 and Table 2. The 24 animals allocated in this study were representative of the target population since there are no reported breed, sex, or age predilections for sarcoptic mange (Curtis, 2004). All dogs presented skin abnormalities and clinical signs consistent with sarcoptic mange infections, which included alopecia, papules, dermatitis, erythema, and crusts.

CERTIFECT® demonstrated a significant acaricidal efficacy from Day 14 post-treatment in the treated dogs. Indeed, results from the mite counts showed that the treated group had significantly ($p<0.05$) lower mite counts compared to the untreated control group at Days 14, 21, 41, and 56 (Table 1). The percent reduction in the number of mites when compared to the untreated

***Table 1.*** *Results of Mite counts by Study Day*

| **Study Day** | **Group 1 : untreated control** Geometric Mean1 (Arithmetic Mean) | **Group 2: CERTIFECT®** Geometric Mean (Arithmetic Mean) | **Reduction** (%) | **P-value[2]** |
|---|---|---|---|---|
| 0 | 19.8 (45.5) | 18.4 (29.1) | NA | NA |
| 7 | 0.7 (3.1) | 0.3 (0.5) | 57.1 | 0.6547 |
| 14 | 3.5 (21.8) | 0 (0) | 100 | 0.0143 |
| 21 | 1.5 (3.1) | 0.1 (0.1) | 93.3 | 0.0143 |
| 28 | 1.8 (8.1) | 0.2 (0.3) | 88.9 | 0.4142 |
| 41 | 2.2 (13.6) | 0 (0) | 100 | 0.0253 |
| 56 | 4.7 (40.8) | 0 (0) | 100 | 0.0143 |

*1 Based on transformation to ln(count+1). There were 11 animals (one was euthanized on Day 5) in the untreated group and 12 animals in the treated group.*
*2 Probability value from Friedman's test.*
*NA = Not Applicable*

control Group was 100%, 93.3%, 100%, and 100% respectively (Table 1). No mites could be found in any CERTIFECT®-treated dogs 14 days following each treatments (ie, Days 14 and 41) and at the end of the study 28 days after the second treatment (ie,, Day 56).

Furthermore the overall treatment success was stated as a marked decrease in the severity and extent of mite-associated clinical signs. The results of this study showed a significant therapeutic effect of CERTIFECT, illustrated by the calculation of the Lesion Scores. Both groups of dogs presented an average Lesion Score of 3 before treatment at the start of the study. From Day 28 until the end of the experiment (ie, Day 56), the group of treated dogs presented significant ($p<0.05$) lower Lesion Scores compared to the untreated control group (Table 2). Whereas the control group remained at a score ranging from 2 to 2.5 throughout the 56 days of the experiment, CERTIFECT®-treated dogs presented a substantial decrease in the Lesion Scores ranging from 0 to 0.5 from Day 35 until the end of experiment. Importantly, the data indicated that treatment resulted in a complete resolution of the skin lesions typical of sarcoptic mange, because by the end of the study the dogs presented almost no skin lesions anymore.

Efficacy of treatments against sarcoptic mange are known to vary considerably; cure rates (based on mite counts and/or clinical signs) can range from 60% to 100% (Wagner and Wendelberger, 2000; Curtis, 2004; Krieger et al, 2005; Fourie et al, 2006). Despite treatments being reported as generally effective for sarcoptic mange control in dogs, some have limitations in their use and others are associated with adverse reactions (Folz, 1984; Griffin, 1993; Plumb, 1995; Curtis, 1996; Wendelberger and Wagner, 1998; Bordeau and Hubert, 2000; Curtis, 2004). In this study, two treatments applied 1 month apart with a spot-on formulation of fipronil/(S)-methoprene plus amitraz resulted in rapid reduction of mite counts and in the cure of clinical signs. No adverse events were observed following the treatment administrations.

This study provided evidence that treatment with CERTIFECT®, applied twice at 28-day intervals, is highly effective against *Sarcoptes scabiei* infection in dogs.

***Table 2.*** *Summary of Lesion Scores by Study Day*

| **Study Day** | **Group 1 : untreated control** Median (Arithmetic Mean) | **Group 2: CERTIFECT®** Median (Arithmetic Mean) | **P-value[1]** |
|---|---|---|---|
| 0 | 3 (2.8) | 3 (2.9) | NA |
| 7 | 3 (2.9) | 3 (2.8) | 1.0000 |
| 14 | 3 (2.9) | 2.5 (2.7) | 0.6547 |
| 21 | 3 (2.9) | 2 (2.3) | 0.1573 |
| 28 | 2 (2.5) | 2 (1.5) | 0.0348 |
| 35 | 2 (2.6) | 0.5 (0.8) | 0.0114 |
| 42 | 2 (2.6) | 0.5 (0.8) | 0.0114 |
| 49 | 2 (2.6) | 0 (0.5) | 0.0114 |
| 56 | 2.5 (2.6) | 0 (0.1) | 0.0027 |

*1 Probability value from Cochran-Mantel-Haenszel test.*
*Lesion Score: 0 no skin lesions*
*1 areas with papules and/or small areas with alopecia, < 4 areas*
*2 areas with papules and/or small areas with alopecia, > 4 areas*
*3 more than half of the body with papules and/or alopecia, crusts present*
*4 chronic infestations with crusts covering more than half the body surface, skin reactions including alopecia or erythema*
*NA = Not Applicable*

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