An open, self-controlled study on the efficacy of topical indoxacararb for eliminating fleas and clinical signs of flea-allergy dermatitis in client-owned dogs in Queensland, Australia

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Introduction
Flea-allergy dermatitis (FAD) is a highly prevalent skin disease of dogs, in which affected individuals experience a hypersensitivity response induced by antigens injected intradermally with the saliva of feeding fleas. Indoxacarb is an oxadiazine insecticide that has been shown to have significant insecticidal activity against the adult cat flea (Ctenocephalides felis), recognized as the most common flea infesting dogs in the USA, Europe and Australia. The insecticidal mechanism of action of indoxacararb is via blockade of neuronal voltage-dependent sodium channels. Following ingestion or contact by fleas, indoxacarb is metabolized by insect esterase enzymes to a highly insecticidal form, a process called bioactivation. In addition to flea adulticide activity, indoxacarb has been shown to have ovicidal activity and to eliminate flea larval stages effectively in the host environment.

Background – Canine flea-allergy dermatitis (FAD), a hypersensitivity response to antigenic material in the saliva of feeding fleas, occurs worldwide and remains a common presentation in companion animal veterinary practice despite widespread availability of effective systemic and topical flea-control products.

Hypothesis/Objectives – To evaluate the clinical response in dogs with FAD treated topically with indoxacarb, a novel oxadiazine insecticide.

Animals – Twenty-five client-owned dogs in Queensland, Australia diagnosed with pre-existing FAD on the basis of clinical signs, flea-antigen intradermal and serological tests.

Methods – An open-label, noncontrolled study, in which all dogs were treated with topical indoxacarb at 4 week intervals, three times over 12 weeks.

Results – Twenty-four dogs completed the study. Complete resolution of clinical signs of FAD was observed in 21 cases (87.5%), with nearly complete resolution or marked improvement in the remaining three cases. Mean clinical scores (Canine Atopic Dermatitis Extent and Severity Index-03) were reduced by 93.3% at week 12. Mean owner-assessed pruritus scores were reduced by 88% by week 12. Mean flea counts reduced by 98.7 and 100% in weeks 8 and 12, respectively.

Conclusions and clinical importance – Topical indoxacarb treatment applied every 4 weeks for 12 weeks, without concomitant antipruritic or ectoparasiticide therapy, completely alleviated flea infestations in all dogs and associated clinical signs of FAD in a high proportion of this population of dogs in a challenging flea-infestation environment.

Materials and methods
This was an open-label, noncontrolled study conducted according to Good Clinical Practice standards and compared mean quantified pretreatment clinical indices with values on three subsequent re-examinations at 4 week intervals. Twenty-five client-owned dogs suspected to have FAD were enrolled in the study with written informed consent of their owners. A relocating owner withdrew one dog after week 4, leaving 24 dogs that completed the study. Results for this dog are included in the week 4 but not the week 8 and 12 calculations. Enrolled dogs were mixed and pure breeds, ranging from 10 months to 12 years old and weighing between 3.2 and 41.6 kg. The initial gender breakdown was as follows: five intact males, 11 neutered males, four intact females and five neutered females (the dog withdrawn from the study was an intact female). The study was conducted during the summer months in Queensland,
Australia (between January and April), when the risk of flea exposure was high. Dogs were kept at home by their owners and fed and exercised according to their usual routine.

The dogs were diagnosed with FAD on the basis of clinical signs consistent with published descriptions of FAD. All dogs were tested for immediate- and delayed-type hypersensitivity reactions to intradermally injected flea antigen (Greer Laboratories, Greer Veterinary, Lenor, NC, USA). Wheal formation at the flea-antigen injection site at 5–10 min (immediate reaction) or 24 h (delayed reaction) postinjection was compared with reactions to the negative (saline) control, maize as a positive irritant control (grade 2+ wheal) and histamine (grade 4+ wheal). A flea-antigen site wheal between grades 2+ and 4+ was classified as consistent with flea-saliva hypersensitivity. If there were no skin reactions then a serum sample was submitted to a commercial laboratory for flea-antibody (IgE) serology testing (Al lercept assay; Heska, Gribbles Veterinary Pathology Laboratory, Clayton, Victoria, Australia). There was a positive immediate-or delayed-type wheal response in 22 of the 25 dogs tested; blood samples from the three negative dogs tested positive for flea antibodies. Additional diagnostic tests for all dogs included skin scrapings, skin cytology and fungal culture; in all cases, these did not reveal any findings that provided an alternative explanation for the observed clinical signs.

Following completion of diagnostic testing, each dog was assessed during weeks 0, 4, 8 and 12 using three parameters as follows.

1. Lesions were measured by a veterinary dermatologist using the Canine Atopic Dermatitis Extent and Severity Index (CADESI-03). For the purposes of analysis, the CADESI-03 results were arbitrarily and empirically converted into the following four nonvalidated categories of increasing severity, i.e. ‘in remission’ or ‘insignificant’, ‘mild’, ‘moderate’ and ‘severe’, based on score intervals of 0–15, 16–59, 60–119 and 120+, respectively. The CADESI scoring system is validated only for assessing clinical signs in dogs with atopic dermatitis.

2. Owners assessed pruritus severity using a score between 0 and 100 obtained from a cross (X) marked on a Pruritus Visual Analog Scale (PVAS).

3. Flea counts were carried out at home using a modified method, where six representative skin areas were searched for fleas for 1 min. If no fleas were found then the entire body was searched for an additional 2 min and if no fleas were seen then the dog was classified as flea free.

Treatment
Following diagnostic testing and baseline disease assessment, the attending veterinarian treated the dog topically with indoxacarb (Activyl; Merck/MSD Animal Health, Summit, NJ, USA) at weeks 0, 4 and 8. The dose was applied to achieve a target dose of at least 15 mg/kg indoxacarb. Treatment was applied at one or more evenly distributed spots along the dorsal midline between the shoulder blades and base of the tail, with larger and heavier dogs receiving more treatment locations.

 Owners were advised not apply any insecticidal treatment to their households or premises during the study period. The dogs received no concomitant treatment with any other flea-control products or with any drug having antipruritic or anti-inflammatory activity, including glucocorticoids, antihistamines and nonsteroidal anti-inflammatory agents, throughout the 12 week study; bathing was permitted. One dog developed bilateral otitis externa and a secondary aural haematoma during the study and received oral cefalexin (14 days), topical otic enrofloxacin and carprofen (3 days); the dog recovered fully. Another dog was diagnosed with a skin and ear infection on the initial study visit and was treated with cefalexin for 21 days and recovered fully.

Each owner gave informed signed consent, and the study was conducted according to Australian Animal Welfare guidelines, with pre-approval from the Queensland Department of Primary Industries and Fisheries: Community Access Animal Ethics Committee prior to commencement.

Statistical analysis
All reported means are arithmetic, and data were transformed as described below to apply appropriate statistical techniques. Significance on statistical tests was declared for P-values <0.05.

Flea count analysis
Flea control efficacy was calculated as the percentage change between each post-treatment and pretreatment flea count using the formula: % Reduction = (1 – (PostTx value ÷ PreTx value)) × 100. Mean live flea counts on day 0 were compared with mean live flea counts at weeks 4, 8 and 12 using generalized linear models (log-linear modelling or regression, also called Poisson regression) for overdispersed Poisson data using the logarithmic link function. This method produces an ‘analysis of deviance’, analogous to the ‘analysis of variance’ for normally distributed data. Observed flea counts were used for the analysis, and the mean-variance relationship was used to link mean counts to the linear model on a logarithmic scale. This analysis was calculated with the GenStat program (GenStat Release 7.2; VSN International Co., Oxford, UK).

Pruritus Visual Analog Scale analysis
Pruritus Visual Analog Scale (PVAS) results are not normally distributed because a disproportionate number of responses can be close to 0, particularly as treated dogs reach their final clinical assessment. Therefore, the nonparametric Wilcoxon signed-rank test for paired samples was used to compare differences in PVAS scores between the following three time points: day 0 and week 4; day 0 and week 8; and day 0 and week 12.

CADESI-03 scores
There were 25 pairs of categorized CADESI-03 scores for the pretreatment and week 4 time points. These were cross-classified to produce a 4 × 4 frequency table. Similar 4 × 4 tables were prepared for the 24 available pairs of scores for the pretreatment and week 8 time points and for the pretreatment and week 12 time points. Bhapkar’s chi-square test for ordered categories, with three degrees of freedom, was used to test overall marginal homogeneity for the two 4 × 4 tables for each pair of time points. A significant result was declared when the sequence of marginal frequencies for the post-treatment categories differed from the sequence for the pretreatment categories to a greater extent than would be expected by chance. Each Bhapkar’s chi-square test was followed by McNemar’s chi-square test of the overall direction of change among the categories. The MH program [User Guide for the MH Program (version 1.2) 2006] was used for these analyses.

Results
All enrolled dogs showed significant improvement in observed clinical signs over the study period. Of the 24 dogs that completed the study successfully, 21 (87.5%) showed complete resolution of their clinical signs based on a CADESI-03 score category of ‘insignificant’ on the final visit. The other three dogs had almost complete resolution or very marked improvement. Some dogs recovered rapidly from clinical signs; 11 dogs (45.8%) had CADESI-03 scores categories of ‘insignificant’ by week 4, and seven additional dogs (29.2%) by week 8. Mean post-treatment CADESI-03 scores at week 4, 8 and 12 revisits were statistically significantly lower than the pretreatment scores (Table 1). No indoxacarb treatment-associated adverse clinical events were observed by owners or veterinarians.
Owner assessment of pruritus decreased significantly ($P < 0.001$) in all dogs over the study period. The mean pretreatment PVAS was 74.5, and this declined to 38.8, 18.7 and 8.9 in weeks 4, 8 and 12, representing reductions of 47.9, 74.8 and 88%, respectively. The lowest PVAS reported at the pretreatment examination was 50.5, while eight owners reported a PVAS of 0.0 at the end of the study period.

The mean pretreatment flea count was 18 per dog; all dogs were classified as flea free at the completion of the 12 week study (Table 2).

### Discussion

This study demonstrated that, in this population, topical treatment of FAD-affected dogs every 4 weeks for 12 weeks with indoxacarb, with no concomitant ectoparasitidal or antipruritic therapy, provided excellent control of clinical signs. All measures, including CADESI-03 scores; owner-assessed pruritus and flea counts, decreased significantly over the study period. The CADESI-03 scoring system is validated only for canine atopic dermatitis and not for FAD; furthermore, the CADESI system cannot be used to classify clinical signs as being definitively caused by FAD; therefore, the classification of the scores into four severity categories was empirical and not based on any formal assessment of the validity of this scoring system for dogs with signs of FAD. For the purposes of this study, it was considered that CADESI-03 provided a useful measurement of the severity of clinical signs. Clinical signs were reduced to ‘insignificant’ in 21 of the 24 enrolled dogs that completed the study. The one dog that did not complete the study had exhibited marked clinical improvement with an ‘insignificant’ CADESI-03 score at the time of withdrawal.

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**Table 1.** Mean pre- and post-treatment CADESI-03 scores of client-owned dogs affected with flea-allergy dermatitis treated topically with indoxacarb three times at 4 week intervals

| Parameter               | Pretreatment | 4 weeks | 8 weeks | 12 weeks |
|-------------------------|--------------|---------|---------|----------|
| Number of dogs          | 25           | 25      | 24      | 24       |
| Mean CADESI-03 score    | 90.1         | 36.9    | 13.5    | 6.0      |
| Percentage reduction from pretreatment | –          | 59.0    | 85.0    | 93.3     |
| P value compared with pretreatment | –          | <0.0001 | <0.0001 | <0.0001 |
Résumé

Contexte – La dermatite par allergie aux piqures de puces (FAD), une hypersensibilité aux antigènes salivaire des puces, est décrite dans le monde entier et reste une présentation fréquente en médecine vétérinaire des animaux de compagnie malgré une large gamme d’antiparasitaires topiques et systémiques efficaces disponibles.

Hypothèses/Objectifs – Estimer la réponse clinique des chiens atteints de FAD recevant de l’indoxacarb topique, un nouvel insecticide de la classe des oxadiazines.

Sujets – Vingt cinq chiens de propriétaires du Queensland, Australie, précédemment diagnostiqués allergiques aux puces à partir des signes cliniques, des tests intradermiques et des tests sérologiques.

Méthodes – Une étude ouverte, non contrôlée, dans laquelle tous les chiens ont été traités avec de l’indoxacarb topique à 4 semaines d’intervalle, trois fois sur 12 semaines.

Résultats – Vingt quatre chiens ont été inclus dans l’étude. Une résolution complète des signes cliniques de FAD a été observée pour 21 cas (87,5%), une résolution presque complète ou une amélioration marquée pour les trois autres cas. Les scores cliniques moyens (Canine Atopic Dermatitis Extent and Severity Index-03) ont été réduits de 93,3% à la semaine 12. Les scores de prurit moyens ont été réduits de 88% à la semaine 12. Les comptages moyens de puce ont été réduits de 98,7% et 100% respectivement aux semaines 8 et 12.

Conclusions et importance clinique – Le traitement topique d’indoxacarb appliqué toutes les 4 semaines pendant 12 semaines, sans antiprurigineux ou ectoparasitaire concomitant, a complètement supprimé l’infestation de puces pour tous les chiens et les signes cliniques associés à la FAD pour une large proportion de cette population de chiens dans un environnement contenant des puces.
Zusammenfassung
Hintergrund – Die Flohspeichelallergie des Hundes (FAD), bei der es sich um eine Hypersensibilitätsreaktion auf das Antigenmaterial im Speichel von saugenden Flöhen handelt, kommt weltweit vor und stellt einen häufigen Vorstellungsgrund in der Kleintierpraxis dar, obwohl weltweit wirksame systemische und topische Flohkontrollprodukte verfügbar sind.

Hypothese/Ziele – Eine Evaluierung der klinischen Antwort von Hunden mit FAD, die topisch mit Indoxacarb, einem neuen Oxadiazin Insektizid, behandelt worden waren.

Tiere – Fünfundzwanzig private Hunde in Queensland, Australien, die mit bereits existierender FAD basierend auf klinischer Symptomatik, positiven Intradermaltests und positiver Serologie auf Flohallergen diagnostiziert worden waren.

Methoden – Eine offene, nicht kontrollierte Studie, in der alle Hunde mit topischem Indoxacarb in 4 wöchigen Intervallen, drei Mal über einen Zeitraum von 12 Wochen behandelt wurden.

Ergebnisse – Vierundzwanzig Hunde beendeten die Studie. Eine gänzliche Abheilung der klinischen Anzeichen von FAD wurde in 21 Fällen beobachtet (87,5%), bei nahezu völlig verschwinden oder einer deutlichen Verbesserung in den restlichen drei Fällen. Die durchschnittlichen klinischen Werte (Canine Atopic Dermatitis Extent and Severity Index-03) waren in der zwölften Woche um 93,3% reduziert. Die durchschnittlichen, von den BesitzerInnen beurteilten, Juckreizwerte waren in der zwölften Woche um 88% reduziert. Die durchschnittliche Anzahl an Flöhen war in den Wochen 8 und 12 um 97,7 bzw 100% reduziert.

Schlussfolgerungen und klinische Bedeutung – Indoxacarb, welches topisch alle 4 Wochen 12 Wochen lang ohne begleitende juckreizstillende Behandlung oder einer Behandlung von Ektoparasiten verabreicht wurde, milderte die Flohinestationen bei allen Hunden und die mit FAD auftretenden klinischen Symptome in einer großen Proportion dieser Hunde­population, die in einer schwierigen Umgebung mit hohem Flohvorkommen lebten.