

Lack of Interference of Oclacitinib with the results of intradermal testing or allergen-specific IgE serology in *Dermatophagoides farinae*-sensitized beagle dogs

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ABSTRACT

Canine atopic dermatitis (AD) is associated with increased levels of allergen-specific IgE due to hypersensitization to environmental allergens. Intradermal testing (IDT) and allergen-specific IgE serology testing are often used to determine the allergens which elicit an IgE response in animals with a diagnosis of AD. The objective of this study was to determine the effects of oclacitinib on IDT and allergen-specific IgE serology testing using a laboratory model of house-dust mite sensitized Beagle dogs. Twenty-four (24) normal, healthy purpose-bred Beagle dogs were sensitized to house dust mites (HDM, *Dermatophagoides farinae*) and randomly assigned to placebo-, oclacitinib- (0.4 mg/kg/dose PO), or prednisolone-treated (0.5 mg/kg/dose PO) groups. After 14 days of twice daily dosing, the effects of prednisolone and oclacitinib were compared to placebo using baseline and post-dose IDT and allergen-specific IgE serum measurements. Sensitized dogs had increased circulating HDM-specific IgE for at least two weeks post-sensitization. Prednisolone significantly inhibited the measurable sensitivity of IDT, while oclacitinib did not. Neither prednisolone nor oclacitinib imposed significant effects on allergen-specific IgE serum levels, suggesting oclacitinib may have potential to be used in dogs concurrently undergoing intradermal skin testing and/or allergen-specific IgE serology testing without interference with test results.

1. Introduction

Canine atopic dermatitis (AD) is a multifactorial disease involving immunological imbalances and is commonly associated with increased levels of allergen-specific IgE (Olivry et al., 1997; Nambi and Kavitha, 2013; McCandless et al., 2014). After a diagnosis of AD is reached, identification of offending allergens may be performed by allergen-specific intradermal testing (IDT) and/or IgE serological testing, with the goal of allergen avoidance (when possible) and identifying relevant allergens to include in allergen-specific immunotherapy (ASIT) (Hensel et al. 2015). Several therapeutic drugs such as antihistamines and glucocorticoids have been shown to interfere with IDT results. Consequently, evidence-based guidelines recommend that antihistamines are withdrawn from patients for 7 days prior to skin testing. Similarly, glucocorticoids should also be withdrawn prior to IDT, however withdrawal times vary based on the type of steroid, route of application and duration of glucocorticoid treatment. In general, it is

recommended that oral and topical glucocorticoids are withdrawn 14 days prior to IDT, and long-acting injectable glucocorticoids for a minimum of 28 days prior to IDT (Olivry et al., 2013). During this withdrawal period, returning clinical signs can be a challenge to manage for the veterinarian, owner, and patient.

Oclacitinib (Apoquel®, Zoetis, Kalamazoo, MI, USA) is an approved first-line treatment for AD and pruritus associated with allergic dermatitis in dogs 12 months of age or older (Apoquel package insert). It has been shown to be effective at rapidly reducing pruritus and improving skin lesions (Cosgrove et al., 2013a; Cosgrove et al., 2013b). Although oclacitinib can effectively manage clinical signs of AD, owners or veterinarians may want to proceed with allergy testing to undergo ASIT. Using a laboratory model of House Dust Mite (HDM)-sensitized beagles, our objective was to determine whether oclacitinib would interfere with IDT and allergen-specific IgE serology results.

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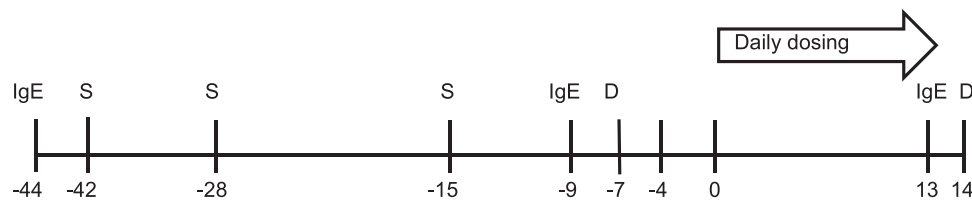


Fig. 1. Study timeline: Activities shown by Study Day (represented by the number below the timeline). Daily treatment dosing occurred Days 0–13. IgE = Blood samples collected for allergen-specific IgE; S = Sensitization via subcutaneous allergen injection; D = Intradermal skin testing.

2. Methods

2.1. Ethical statement

Care of all animals used in this study was in accordance with the Zoetis IACUC guidelines. This study was conducted in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures, and in accordance with local laws and regulations.

2.2. Animals

Twenty-four purpose-bred adult beagle dogs from Marshall Bio-Resources (North Rose, NY, USA) were used in this study. Twelve neutered males and 12 spayed females were single-housed in metabolism cages. All study activities were performed in each animal's home kennel.

2.3. HDM sensitization

Dogs were administered three subcutaneous injections of allergen at two-week intervals (Days –42, –28 and –15, Fig. 1). Each injection contained 10 µg of the HDM allergen (*Dermatophagoides farinae*, Greer Laboratories, Lenoir, NC, USA) plus 2 mg of Rehydralgel (aluminum hydroxide gel, Reheis Inc, Berkeley Hts, NJ, USA) in a mixture with sterile phosphate buffered saline.

2.4. Intradermal testing

To help visualize the wheal and flare reaction, dogs were first injected intravenously with 4 mL of 1.0 % solution of sterile Evans Blue Dye. Each animal then received 8 intradermal injections representing six 10-fold serial dilutions of *D. farinae* (from 0.0005 µg to 50 µg protein), one injection of histamine (0.125 µg; positive control), and one injection of phosphate buffered saline (negative control). Each injection was given in a volume of 100 µL. Fresh dilutions were made from lyophilized *D. farinae* for each skin testing day. The hair coat over the lateral thorax was shaved with an adjustable clipper blade. Injections were delivered using 0.5 mL syringes with 27-gauge needles. Injection sites were identified with a waterproof marker. Fifteen minutes after injection, the maximal diameter and its perpendicular diameter were measured in millimeters and averaged. The wheal was considered a positive response if the average of the two dimensions was greater than or equal to the average of both dimensions of each of the positive and negative control measurements. The lowest positive dilution from IDT was determined at baseline (Day –7) and post-dose (Day 14) for each animal. A change greater than or equal to 2-log change was considered significant. “NA” was recorded if the positive control (histamine) did not result in a measurable wheal, or if no measurable wheal resulted from any *D. farinae* dilutions.

2.5. Study design

Baseline skin tests were performed on Day –7, and HDM-specific titers were determined before and after the sensitization (Days –44 and –9 in Fig. 1).

This study was a parallel group, randomized block design. Each block contained animals from each of the three treatments. This study was conducted as a single center, masked, randomized controlled trial. Twenty-four HDM-sensitized beagles were randomized to three treatment groups based on Day –7 IDT data. Each group of eight dogs was dosed twice daily (12 ± 2 h between doses) for 14 consecutive days starting on Day 0 with either placebo (control placebo caplets, Zoetis, Kalamazoo, MI, USA), oclacitinib at 0.4 mg/kg/dose (Apoquel, Zoetis, Kalamazoo, MI, USA, 2022), or prednisolone at 0.5 mg/kg/dose (PrednisTab, Lloyd Inc, Shenandoah, IA, USA). HDM-specific IgE titers were examined again after the morning treatment administration on Day 13, and intradermal skin testing was performed on Day 14 (see Fig. 1.).

2.6. Sample collections

Blood samples for allergen-specific IgE levels were collected via the jugular vein into serum separator tubes (BD Vacutainer® SST™). Samples were allowed to clot for approximately 30 min. Samples were then centrifuged, and the serum was frozen and stored in 96-well plates until analysis (Zoetis, Kalamazoo, MI).

2.7. Allergen-specific IgE levels

Allergen-specific IgE levels were assessed in serum using an ELISA-based immunoassay. *Dermatophagoides farinae* (8 µg/mL in phosphate buffered saline) was coated onto 96-well Maxisorp ELISA plates (Thermo Fisher) to capture allergen-specific IgE in serum onto the plate. After 12–24 h incubation time at 2–8°C, coating buffer was removed by flicking plate in a sink without washing. Sucrose (1 %) blocking buffer was added to each well and plates were incubated at room temperature for at least 2 h. Plates were washed with 0.05 % Tween-20 in 1X phosphate buffered saline (1X PBST) wash buffer. Study sample serum was initially diluted 1:50 with assay diluent (100 mM Tris 1 % BSA pH7.5), further 1:2 serial dilutions were made with a final dilution of 1:1600. Diluted samples were added to the plate in duplicate and incubated for one hour with shaking. Plates were washed with 0.05 % Tween-20 in 1X phosphate buffered saline (1X PBST) wash buffer. Biotinylated monoclonal anti-dog IgE detection antibody (1 µg/mL, Immunology Consultants Laboratory) in assay diluent (100 mM Tris with 1 % BSA) was added and incubated at room temperature for 1 h. Plates were then washed again with the 1X PBST buffer, followed by Streptavidin HRP in assay diluent (100 mM Tris with 1 % BSA). Plates were washed with 1X PBST and One-Step TMB Reagent (Thermo Scientific) was added, followed by a 30-minute incubation at room temperature and finally, Stop Solution (1 N HCL, Fisher Scientific) was added. Optical density at 450 nm was determined using a SpectroMAX Plus (Molecular Devices). Values represent mean serum dilutions that produce a signal 3 standard deviations above background ($n = 8$).

2.8. Statistical analyses

IDT Data - For each animal, skin test sensitivity (lowest positive dilution) on Day 14 was compared to the baseline (Day –7) measurement. If there was at least a 2-log decrease from the baseline (Day –7) measurement, it was considered a “decrease.” Frequency distributions of

Table 1

The average axis measurement for each IDT positive and negative control.

Treatment	Animal #	Day – 7 negative control (mm)	Day – 7 positive control (mm)	Day 14 negative control (mm)	Day 14 positive control (mm)
Placebo	1	0	5	0	5.5
	2	0	5.5	0	8.5
	3	0	4	0	8
	4	0	3.5	0	6
	5	0	7	0	9.5
	6	0	3.5	0	8
	7	0	3	0	8
	8	0	3	0	8
oclacitinib	9	0	7	0	9
	10	0	5.5	0	7.5
	11	0	6.5	0	5
	12	0	3	0	0
	13	0	5.5	0	5
	14	0	3	0	5
	15	0	2	0	9.5
	16	0	2.5	0	8
Prednisolone	17	0	5.5	0	5.5
	18	0	4	0	0
	19	0	4.5	0	0
	20	0	6.5	0	0
	21	0	8.5	0	8
	22	0	5.5	0	6
	23	0	6	0	0
	24	0	4.5	0	8

Table 2

IDT sensitivity changes from baseline for individual animals by treatment.

Treatment	Animal #	Day – 7 Baseline effect (μg)	Day 14 post-treatment effect (μg)	Significant change in sensitivity
Placebo	1	0.05	0.05	No change
	2	0.05	0.05	No change
	3	0.0005	0.05	2-log
	4	0.005	0.05	No change
	5	0.005	0.005	No change
	6	0.05	0.05	No change
	7	0.05	0.05	No change
	8	0.005	0.5	2-log
oclacitinib	9	0.005	0.05	No change
	10	0.005	0.05	No change
	11	0.5	0.05	No change
	12	0.05	0.05	No change
	13	0.05	0.05	No change
	14	0.05	0.5	No change
	15	0.005	0.05	No change
	16	0.0005	0.05	2-log
Prednisolone	17	0.0005	0.05	2-log
	18	0.5	NA	Loss of sensitivity
	19	0.05	NA	Loss of sensitivity
	20	0.005	NA	Loss of sensitivity
	21	0.005	50	4-log
	22	0.05	0.5	No change
	23	0.005	NA	Loss of sensitivity
	24	0.05	NA	Loss of sensitivity

The lowest positive dilution of HDM is recorded. Greater than or equal to 2-log reduction in sensitivity from baseline is considered a significant sensitivity reduction. "NA" was recorded if the positive control (histamine) did not result in a measurable wheal, or if there was no measurable wheal to the *D. farinae* dilutions.

"decreased"/"did not decrease" were calculated for each treatment. This new variable was analyzed with a generalized linear mixed model for repeated measures with a logit link function. The fixed effects in the model were treatment, time point and treatment by time point

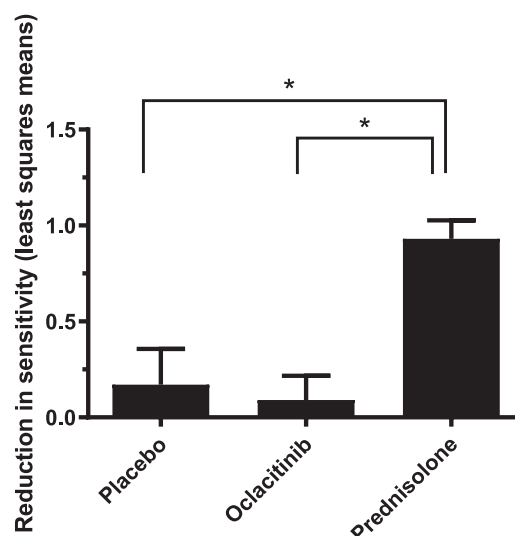


Fig. 2. Intradermal testing was performed on Day – 7 (baseline) and Day 14 to determine minimum positive dilutions. Changes in sensitivity after dosing (Day 14) were determined and plotted as least squares means $\pm$ SEM ($P \leq 0.0290$).

interaction. The random effects in the model were room, block within room, treatment by block within room (animal term). If the treatment and/or the treatment by time point interaction was significant, then all possible pair-wise treatment comparisons were made at each time point. If it was not possible to use the generalized linear mixed model, then each time point was analyzed using Fisher's exact test to test for the significance overall treatment effect, and also to test for pairwise differences between treatments if the overall test for the treatment effect was significant.

HDM-specific IgE levels – HDM-specific IgE was logarithmically transformed prior to analysis. The transformed values were analyzed with a general linear mixed model for repeated measures. The fixed effects in the model were treatment, time point and treatment by time point interaction. The random effects in the model were room, block within room, treatment by block within room interaction (animal term) and residual.

Treatment least squares means, 90 % confidence intervals, minimums and maximums were calculated for each time point. The treatment least squares means and confidence intervals were back-transformed for presentation. If the treatment or treatment by time point interaction is significant, then all possible pair-wise treatment comparisons were made at each time point.

3. Results

3.1. IDT

The average axis measurement for each IDT positive and negative control is listed in Table 1. Of the five prednisolone-treated dogs which exhibited loss of measurable sensitivity, four had no response to the histamine positive control at day 14. A fifth dog had no measurable wheal to any HDM dilutions administered (Table 2), which would have affected the interpretation of even a qualitative IDT assessment.

Two of the eight placebo-treated animals and one of eight oclacitinib-treated animals exhibited a significant reduction in sensitivity compared to their baseline values. All other placebo- or oclacitinib-treated dogs demonstrated no significant change at Day 14 when compared to baseline. Seven of eight prednisolone-treated dogs had significant reductions on Day 14 sensitivity compared to baseline; in two there was at least a 2-log decrease while five dogs exhibited a loss of measurable sensitivity (Table 2).

Sensitivity of the prednisolone-treated group to IDT was significantly

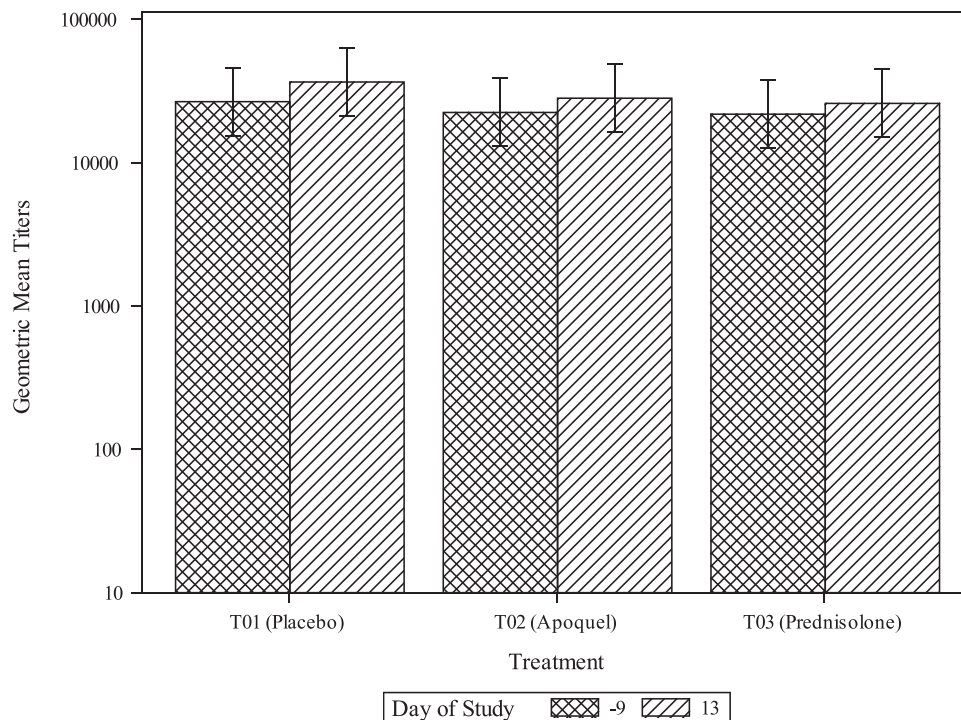


Fig. 3. HDM-Specific IgE Titers. Geometric means with 90 % confidence limits are shown by assigned treatment post-sensitization/pre-treatment (Day –9) and post-treatment (Day 13). HDM-specific IgE titers remain elevated in all treatment groups on Day 13. There were no significant differences in HDM-specific IgE titers between treatments after a 2-week dosing period.

reduced when compared to both the placebo group ($P = 0.0290$) and the oclacitinib group ($P = 0.0233$) (Fig. 2). There was no significant difference in sensitivity reduction between placebo and oclacitinib-treated groups ($P = 0.6744$).

3.2. HDM-specific IgE levels

IgE levels were measured after sensitization to *D. farinae* but before drug treatment on Day –9, and then after treatment administration on Day 13. Mean HDM-specific IgE levels for both days are shown by assigned treatment group in Fig. 3. Mean titers remained elevated after two weeks of treatment administration (Day 13). There were no significant differences in HDM-specific IgE titers between treatments on Day 13 (Fig. 3).

4. Discussion

In this study, we have utilized a laboratory model of allergen hypersensitivity where beagle dogs sensitized to HDM demonstrated a positive skin test reaction and an increase in HDM-specific IgE in serum after HDM sensitization, a common offending allergen in dogs with atopic dermatitis (Kang et al., 2014). This model allowed us to objectively study the potential of current therapeutics to interfere with IDT and allergen-specific IgE serology. Advantages of studying effects in a laboratory model are: 1) use of a uniform population of animals and a single allergen to help reduce variability of the responses, 2) objective scoring and repeated measurements in the same animal to allow for quantitative assessments, and 3) inclusion of a placebo arm comparator to help with interpretation of results.

The use of a uniform population of purpose-bred beagle dogs within a research facility allowed for controlled exposure to the allergen being evaluated, and reduced exposure to other offending allergens present in “at home” settings. *D. farinae* was selected as the sensitizing allergen for this study due to its prevalence in canine allergy and positive response in IgE testing (Kang et al., 2014). Use of a single allergen in the

sensitization and testing of the study dogs eliminated much of the variability which may be seen in pet dogs with spontaneous disease. Importantly, this controlled study design which utilized a consistent protocol within a uniform population of dogs allowed for clear, meaningful data which can be extrapolated into the atopic patient.

Secondly, an objective scoring method was implemented to measure the response to allergen in the skin. Wheals were measured 15 min after allergen exposure. The maximal diameter of the wheal and its perpendicular diameter were measured and averaged. The wheal was considered a positive response if the average of the two dimensions was greater than or equal to the average of the four control measurements. While subjective scoring of IDT is commonly used in clinical veterinary dermatology (Hubbard, White, 2011), we chose an objective methodology to compare *D. farinae* sensitivity results from pre- and post-dose testing. A single log change in sensitivity may occur with natural variability in skin reactivity over time. A two-log change in sensitivity is a more substantial, clinically relevant change. Also worth noting is that the sheer number of allergens used by a dermatologist in an IDT contributes to the time needed to assess wheal reactions (Hubbard, White, 2011). The IDT in this study was limited to eight total injections which decreases the time window needed to assess reactions and thus increases consistency of the results.

Finally, including a placebo arm allowed for determination of whether the change in sensitivity was due to drug influence or simply variability in test results over time or among animals. The negative placebo group remained sensitive to HDM throughout the study, with two of the dogs showing decreased sensitivity to the allergen. In contrast, the prednisolone-treated dogs showed a significant reduction in sensitivity to the allergen with IDT.

While we have expanded upon the advantages of this model, we recognize that the use of a single allergen in a population of laboratory beagle dogs which is not pre-disposed to atopic conditions does not capture the complexity of an atopic animal. An atopic dog in an “at home” setting may differ in breed, genetics, and housing, and may also be exposed to a broader number of potential allergens at varying

concentrations and for varying lengths of time. Therefore, additional studies should be performed in client-owned animals to confirm these findings seen in laboratory beagle dogs.

Changes in IDT reactivity observed in this study with oral prednisolone have also been observed in other controlled laboratory dog studies, as well as in client-owned dogs with naturally occurring AD (Olivry, Saridomichelakis, 2013). Therefore, the International Committee on Atopic Diseases of Animals (ICADA) recommends up to a 14-day withdrawal period before IDT assessments, which may allow for clinical signs of AD to reappear. Prednisolone had no significant effect on circulating HDM-specific IgE titers in this study, which is similar to other published studies (Olivry, Saridomichelakis, 2013). Neither IDT nor allergen-specific IgE are standardized, and discrepancies between results from the two assays have been observed during diagnostic workups (Hubbard, White, 2011). False negatives are estimated to occur in up to 30 % of IDT testing and may be attributed to prolonged time from last allergen exposure, and variable techniques, allergen concentrations and sources between practitioners, or a patient affected by atopic-like (non-IgE mediated) disease (Hubbard, White, 2011; McCall et al., 2001; Hillier, DeBoer, 2001). Drug effects in the skin that are not occurring in circulation may also contribute to muted IDT results. To produce a wheal within the skin, allergen binds allergen-specific IgE bound to mast cells, inducing a phenotypic change within the cells and release of inflammatory mediators such as histamine in the skin that can lead to the production of a wheal (Olivry et al., 2001). Circulating allergen-specific IgE is produced from B-cells. Drugs like prednisolone may not alter the ability of B-cells to produce allergen-specific IgE, which could explain the difference in effects on IDT and circulating allergen-specific IgE.

Intradermal testing in dogs is generally performed by veterinary dermatologists in the specialty hospital setting. Positive results do not necessarily indicate a clinically-relevant hypersensitivity (Hubbard, White, 2011). However, after the AD diagnosis is made, IDT results can assist in identifying allergens to avoid, or include in ASIT therapy. Our findings showed that oclacitinib did not cause any significant changes compared to placebo control in skin testing results. Furthermore, oclacitinib had no significant effect on circulating HDM-specific IgE titers, suggesting oclacitinib may not interfere with intradermal or serum testing when determining sensitivity to allergens. Although these study results are encouraging, additional studies in client-owned animals are needed to confirm these findings.

In some atopic dogs, it has previously been a challenge to withdraw anti-pruritic therapy for an adequate time period to be able to perform allergy testing. Withdrawal of anti-pruritic therapy can result in a decrease in the quality of life of the patient, disruption of the pet-owner bond and increase the caregiver burden. It is therefore essential to identify any impact anti-pruritic therapy may have on IDT or allergen-specific IgE serology results. Neither steroids nor oclacitinib influenced HDM-specific serology in this study. However, animals should be weaned from steroids prior to performing IDT, which is consistent with recommendations by ICADA (Olivry, Saridomichelakis, 2013). Fortunately, patients can experience relief from itch during IDT with

oclacitinib, as this study has demonstrated its use does not interfere with either IDT or allergy specific IgE serology test results under the conditions of this study.

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References

- Apoquel (oclacitinib) [package insert], 2022. U.S. Food and Drug Administration website. (https://www.zoetis.com/products/dogs/apoquel/downloads/final_apoquel_pi_030116.pdf).
- Cosgrove, S.B., Wren, J.A., Cleaver, M.D., Martin, D.D., Walsh, K.F., Harfst, J.A., Follis, S.L., King, V.L., Boucher, J.F., Stegemann, M.R., 2013a. Efficacy and safety of oclacitinib for the control of pruritus and associated skin lesions in dogs with canine allergic dermatitis. *Vet. Dermatol.* 24 (5), 479–3114.
- Cosgrove, S.B., Wren, J.A., Cleaver, M.D., Walsh, K.F., Follis, S.L., King, V.L., Jezaniah-Kira, S.T., Stegemann, M.R., 2013b. A blinded, randomized, placebo-controlled trial of the efficacy and safety of the Janus kinase inhibitor oclacitinib (Apoquel®) in client-owned dogs with atopic dermatitis. *Vet. Dermatol.* 24 (6), 587–597 e141–2.
- Hensel, P., Santoro, D., Favrot, C., Hill, P., Griffin, C., 2015. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Vet. Res.* 11, 196.
- Hillier, A., DeBoer, D.J., 2001. The ACVD task force on canine atopic dermatitis (XVII): intradermal testing. *Vet. Immunol. Immunopathol.* 81, 289–304.
- Hubbard, T.L., White, P.D., 2011. Comparison of subjective and objective intradermal allergy test scoring methods in dogs with atopic dermatitis. *J. Am. Anim. Hosp. Assoc.* 47 (6), 399–405.
- Kang, M.H., Kim, H.J., Jang, H.J., Park, H.M., 2014. Sensitization rates of causative allergens for dogs with atopic dermatitis: detection of canine allergen-specific IgE. *J. Vet. Sci.* 15 (4), 545–550.
- McCall, C., Hunter, S., Stedman, K., Weber, E., Hillier, A., Bozic, C., Rivoire, B., Olivry, T., 2001. Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet. Immunol. Immunopathol.* 78 (3–4), 231–247.
- McCandless, E.E., Rugg, C.A., Fici, G.J., Messamore, J.E., Aleo, M.M., Gonzales, A.J., 2014. Allergen-induced production of IL-31 by canine Th2 cells and identification of immune, skin, and neuronal target cells. *Vet. Immunol. Immunopathol.* 157, 42–48.
- Nambi, A.P., Kavitha, S., 2013. Canine atopic dermatitis. *Intas Polivet* 14 (2), 236–240.
- Olivry, T., Saridomichelakis, M., 2013. Evidence-based guidelines for anti-allergic drug withdrawal times before allergen-specific intradermal and IgE serological tests in dogs. *Vet. Dermatol.* 24 (2), 225–e49.
- Olivry, T., Naydan, D.K., Moore, P.F., 1997. Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. *Am. J. Dermatopathol.* 19 (5), 477–486.
- Olivry, T., Dunston, S.M., Murphy, K.M., Moore, P.F., 2001. Characterization of the inflammatory infiltrate during IgE-mediated late phase reactions in the skin of normal and atopic dogs. *Vet. Dermatol.* 12, 49–58.