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Nucleotide supplementation as a novel adjunctive therapy for canine atopic dermatitis[1]

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ABSTRACT.- Barroso C.D.N., Cavalheiro V.L., Imamura L.M., Cordeiro C.C., Klassen E., Gadotti A.C., Ingberman M., Caron L.F., Costa M.C. & Beirão B.C.B. **Nucleotide supplementation as a novel adjunctive therapy for canine atopic dermatitis.** *Pesquisa Veterinária Brasileira 46:e07788, 2026.* Department of Veterinary Pathology, University Federal of Paraná, Curitiba, PR, Brazil, CEP 81531-980. E-mail: breno.beirao@ufpr.br

Atopic dermatitis (AD) is an important allergic condition in dogs, and treatment is lifelong. Alternatives for therapy are important, as dogs do not respond equally to commonly used therapies. Novel therapies on the market are also costly, which limits their adoption in low and middle-income countries. Nucleotides have been shown to improve innate barrier function. Polynucleotides, in particular, have long been studied for skin heath by promoting cellular proliferation and reducing inflammation. Therefore, we tested nucleotides for their efficacy in canine atopic dermatitis. Two independent mouse trials were performed. The first trial (pilot) aimed to standardize the atopic dermatitis challenge model and to perform a proof-of-concept for the use of nucleotides for the treatment of AD. The second trial expanded on the results of the first by assessing the oral use of the experimental compound. Lastly, a clinical trial was run on 27 dogs with naturally occurring AD, for 8 weeks. The mouse trials revealed that use of nucleotides diminished the dermal inflammation that is common in AD. On histopathological analyses, scores of inflammatory cells, and fibrosis in the dermis of were lower in treated mice, resulting in diminished dermal thickness. Both oral and injectable administration routes proved effective. Differently from what occurred in the dermis, epidermal thickening was not reversed by the test compound. In dogs, oral treatment reduced owner-assessed pruritus and in vet-assessed erythema. These data indicate a possible use for oral nucleotide supplementation against canine AD.

INDEX TERMS: Atopic dermatitis, canine, allergy, pruritus, nucleotides, mouse model.

RESUMO.- Nucleotide Supplementation as a Novel Adjunctive Therapy for Canine Atopic Dermatitis A dermatite atópica (DA) é uma importante condição alérgica em cães, cujo tratamento é vitalício. Alternativas terapêuticas são relevantes, pois os cães não respondem de maneira uniforme às terapias comumente utilizadas. Além disso, novas terapias disponíveis no mercado apresentam custos elevados, o que limita sua adoção em países de baixa e média renda. Os nucleotídeos demonstraram-se úteis em melhorar a função de barreira inata. Os polinucleotídeos, em especial, têm sido estudados há muito tempo por seus benefícios à saúde da pele, promovendo a proliferação celular e reduzindo a inflamação. Portanto, testamos a eficácia dos nucleotídeos na dermatite atópica canina. Dois experimentos independentes em camundongos foram realizados. O primeiro (piloto) teve como objetivo padronizar o modelo de indução de dermatite atópica e realizar uma prova de conceito para o uso de nucleotídeos no tratamento da DA. O segundo experimento ampliou os resultados do primeiro,

avaliando o uso oral do composto experimental. Por fim, foi conduzido um ensaio clínico em 27 cães com DA naturalmente adquirida, durante 8 semanas. Os estudos em camundongos revelaram que o uso de nucleotídeos reduziu a inflamação dérmica característica da DA. Nas análises histopatológicas, os escores de células inflamatórias e de fibrose na derme foram menores nos animais tratados, resultando em redução da espessura dérmica. Tanto a administração oral quanto a injetável mostraram-se eficazes. Diferentemente do observado na derme, o espessamento epidérmico não foi revertido pelo composto testado. Em cães, o tratamento oral reduziu o prurido avaliado pelos tutores e o eritema avaliado pelos veterinários. Esses dados indicam um possível uso da suplementação oral com nucleotídeos no controle da dermatite atópica canina.

TERMOS DE INDEXAÇÃO: Dermatite atópica, canina, alergia, prurido, nucleotídeos, modelo murino.

INTRODUCTION

Canine atopic dermatitis (CAD) is one of the most prevalent dermatological conditions in dogs. It is an allergic disease with a genetic predisposition for excessive IgE responses to innocuous stimuli (Halliwell, 2006; Lian & Halliwell, 1998). CAD occurs with overt inflammation and pruritus that occur due to exacerbated immune responses, mainly derived from CD4+ T helper 2 (TH2) lymphocytes. Activated T CD4+ cells releasing a complex cytokine array, involving various cytokines irregularly between cases. For instance, TH2 cytokines, such IL-4, IL-5, and IL-13, contribute to inflammation and development of injuries which commonly are located on the rostrum, axillary and inguinal regions, abdomen, and extremities of dogs, although this is not consistent in all reports (Brandt, 2011; Hensel et al., 2015; Jeong et al., 2003; Nuttall et al., 2019). IL-31 has recently become recognized as being especially relevant to pruritus (Tamamomo-Mochizuki et al., 2023).

No curative treatment is available for CAD, but some medications effectively reduce pruritus and severity of skin lesions. Glucocorticoids, cyclosporin, antihistamines, monoclonal antibodies lokivetmab and tirnovetmab, small-molecule inhibitors oclacitinib, atinvicitinib and ilunocitinib, have been used. Some treatments show variable efficacy and important adverse effects. Treatment side effects may include vomiting, diarrhoea, alterations in bodyweight and immunosuppression (Elkholly et al., 2020; Olivry et al., 2015, Foster et al., 2025; Wichtowska and Olejnik, 2025; *Elanco press release*). Pricing of treatment is also a problem, especially in low and middle-income countries, affecting the accessibility to some of the therapies (anecdotal report). Given the wide prevalence of the disease, its clinical importance, and chronicity, alternatives are needed to alleviate clinical signs.

Nucleotide supplementation has widely been used in livestock with the goal of improving intestinal health and of modulating immunity by providing such nutrients for the rapid proliferation of immune and mucosal cells (Jung & Batal, 2012; Peng et al., 2013; Superchi et al., 2012). Nucleotides have been shown to have positive effects in immune mediated conditions, including atopic dermatitis (Bae et al., 2026; Dancey et al., 2006). Nucleotide supplementation can serve as the buiding blocks of DNA and RNA, but larger polynucleotides can also directly alter the immune phenotype via specific receptors (Bae et al., 2026; Ha et al., 2025). Polynucleotides are composed of longer lengths, of variable size, but at least bigger than tens of nucleotide bases. Polynucleotides have been studied in skin health for their capacity in activating different immune pathways in relation to single nucleotides, since they can interact with other receptors (Cleaves, 2023; Pitassi et al., 2024). Therefore, the goal of this study was to test oral supplementation of long nucleotides as possible adjunctive in the treatment of CAD. For this, we conducted mouse trials with experimentally-induced AD and a clinical trial in the target species, dogs with naturally occurring disease.

MATERIALS AND METHODS

Ethical approval. (All animal work was evaluated and approved by the Ethics Committee for Animal Research of Federal University of Paraná (protocol number 068/2017) and of Imunova Análises Biológicas LTDA (002.2018). Mice were maintained in line with good husbandry practices (Foster et al., 2014). Dogs were clinically assessed by a veterinarian and were maintained with their owners [guardians] throughout the trial. Written informed consent was obtained from the dog guardians, who were instructed on the intervention, on their own role in decision-making, on the alternative treatments and on the possible risks of the trial.

Mouse trials. Two independent mouse trials were performed. The first trial (pilot) aimed to standardize the atopic dermatitis challenge model and to perform a proof-of-concept for the use of nucleotides for the treatment of CAD. The second trial expanded on the results of the first by assessing the best route to deliver the experimental compound. To induce atopic dermatitis in mice, 1% of 2-chloro-1,3,5-trinitrobenzene (TCNB) was used diluted in acetone, according to the literature (Giménez-Rivera V.A., Metz M., 2014; Kitagaki et al., 1995) . TCNB is a hapten molecule commonly used in animal contact hypersensitivity model since it is a potentially allergenic substance that binds to proteins, eliciting an immune response. Thus, the animals received 20 µL of

TCNB both on the internal and external faces of the right ear pinna. In the first week, it was administered on D1 and D7, and later every other day for 35 days.

Four experimental groups of mice were used for each trial. In both, the negative control only received acetone (as the hapten carrier control). The positive control was the hapten challenge control for induced AD, but was not treated. In the first trial, one group was treated with betamethasone, an anti-inflammatory, as a treatment control. Dosage of nucleotides was selected as previously described for mice (Novak et al., 1994) . The second trial evaluated nucleotide oral and injectable delivery along with an empirical dose reduction and the addition of the carrier aluminium hydroxide ($AlOH_3$) to one of the formulations as it has been shown to protect and slowly release nucleotides, improving its absorption (Nakayama et al., 2010). The experimental design and further information for each trial are presented in Table 1.

At the end of the study, mice were euthanized. Ears were sampled for histology after 50 days (first trial) and 40 days (second trial) of the first application of TCNB. Histological sections were blindly evaluated by an independent pathologist for histopathologic features (ear swelling, leucocyte infiltration and degenerative patterns).

Dog clinical trial. From October to December of 2020, dogs were selected for the trial based on dermatological evaluation and presumptive AD diagnosis as described previously. There was not a minimum score of pruritus or clinical severity for enrolment, but rather we sought to fulfil Favrot's criteria for CAD diagnostics (Hensel et al., 2015) . Other pruritic diseases were ruled out clinically based on consistent history and physical examination alone. All dogs had also been diagnosed with AD by previous attending veterinarians. These animals had previously failed to respond to the common therapies or that were withdrawn from them due to adverse effects. A withdrawal period of 30 days for glucocorticoids, antibiotics, cyclosporin and other AD treatments was required before the trial.

The test compound was an aqueous formulation of polynucleotides (proprietary composition of Imunova Análises Biológicas LTDA.). The product can be provided by the authors for replication or further studies upon reasonable request. Two hundred microliters (200 µg) of nucleotides were used for each dose. The compound was administered orally with a 1 mL syringe by a veterinarian at the day of enrolment and then again 14 days later. Dogs were assessed by owners with a visual analogue pruritus scale ("enhanced scale" of Hill et al., 2007) . The veterinarian also assessed the dogs in the clinic using the CADESI-03 scale (Olivry et al., 2007) . Assessments were performed 14 days after each dose. The trial was ended following the clinical assessment of the second dose.

Statistics. Data distribution was assessed by the D'Agostino-Pearson omnibus normality test. Mouse ear pinnae were compared by unpaired t test with Welch´s correction (dermis) or one-way ANOVA (epidermis). Lesion scores of dogs were compared with Kruskal–Wallis test. P was attributed at 0.10 (Amrhein et al., 2017; Dahiru, 2008) . GraphPad Prism 8 (GraphPad, Inc.) was used for statistics and for graphs.

RESULTS

Mouse trials

The repeated topical applications of TCNB induced a clinical condition on mouse pinnae that was similar to canine atopic dermatitis in both trials, as had been previously described (Kitagaki et al., 1995) . In the pilot study, as expected, challenged ears became visibly sensitive and with evident erythema and thickening (Fig.1A and 1B). The experimental challenge induced dermal thickening due to fibrosis, congestion, diffuse dermatitis with abundant neutrophils and fibroblasts, angiogenesis, and dermal edema, as assessed by a trained veterinary pathologist (descriptive data). The test product reverted the effect of dermatitis. The effect of the trial product was visually comparable to those seen with betamethasone, reducing dermis width in relation to the challenged control, as illustrated in Fig.1B. No statistical analyses were possible in the pilot trial due to the low number of animals.

The second mouse trial assessing the use of the nucleotides compared administration via the oral route *vs* injectable. The second trial showed that dermis width was reduced with oral treatment in the presence of aluminium hydroxide. However, the lower dose of injected nucleotides (without $AlOH_3$, T1) used in the second mouse trial was not sufficient for controlling dermal oedema (Fig.1C). In the second trial, the treatment was only effective in the presence of $AlOH^3$, both orally and via the parenteral route. In these circumstances, the experimental treatment significantly reduced dermal thickness. Epidermal thickening could not be reverted by any of the mouse treatments, however (Fig.1C).

Dog clinical trial – description of enrolled dogs

Out of 350 dogs enrolled, 27 fulfilled the inclusion criteria. 14/27 were female. Mongrel dogs were the most common (n = 8/27), followed by Lhasa Apso (n = 6/27) and Shitzu (n = 4/27). Mean age and weight were 6 ± 3 years and 8 ± 11.6 kg, respectively. 20/27 dogs had previously received corticosteroids and/or cyclosporin for the treatment of AD, and 17/20 were responsive the these.

Dog clinical trial - treatment

Pruritus was the main clinical complaint at study enrolment for all dogs. Excoriations on the skin were commonly seen on the inguinal region, abdomen, limb extremities and ear pinnae. All dogs had detectable erythema (Fig.2A). 37% of animals had chronic otitis. None of the dogs presented with secondary cutaneous bacterial or yeast infections.

Veterinarian-assessed erythema and owner-assessed pruritus were reduced by the experimental treatment after 2 and 1 doses respectively (Fig.2A, B). Alopecia and excoriation were not significantly improved by the treatment (Fig.2A). Only 3 animals showed possible side effects of the experimental treatment. Fever (n = 1 animal), reduced activity (n = 1) and intestinal constipation (n = 1) were seen throughout the experiment, although their association to the treatment was not further investigated, since these clinical signs were transitory.

DISCUSSION

Canine AD is a disease manifested as chronic, pruritic, and inflammatory dermatitis, which requires long-term treatment. Immunologically, it is characterized by a complex lymphocyte response, which coordinates IL-4, IL-5, IL-13, IL-31 release, leading to IgE production in acute syndromes, with a progression toward TH1 cytokines in chronic conditions (Gedon & Mueller, 2018; T. Nuttall et al., 2013; Prelaud & Laprais, 2020; Tamamomo-Mochizuki et al., 2023). Cytokine release, neuronal itch stimuli and the pruritic behaviours of the animal establish a vicious cycle of itch that perpetuates and potentially exacerbates the skin lesions and defects in the skin barrier function (Marsella et al., 2012; Nagafuchi et al., 1997) .

The manifestations of AD described here for TNCB-sensitized mice were caused by recurrent exposure to the chemical, which is a potent hapten allergen, resulting in chronic inflammation of the ear skin. The TNCB-induced mouse model of atopic dermatitis induced in our work was established elsewhere (Kitagaki et al. 1995, 1997; Burns et al. 2005). The model induces T-cell based hypersensitivity with an evolution dynamics that covers both TH1 and TH2 responses, which is also the case for the canine atopic dermatitis (Tamamoto-Mochizuki et al. 2024). Following a hapten challenge, T cells infiltrate the application site and produce cytokines that mediate the characteristic tissue oedema (Kish et al., 2011). This perturbation also develops barrier and epidermal abnormalities, consequently generating the typical histopathological profile (Man et al., 2008).

The mice in our study showed significant ear thickening. Dermal thickening was reverted by both oral and injectable nucleotide treatment in the presence of $AlOH_3$. We had anticipated that $AlOH_3$ would be beneficial as it delays nucleotide uptake, probably elongating the effects of the test product in time (Nakayama et al., 2010).. It remains to be tested whether larger concentrations of free nucleotides, in the absence of $AlOH_3$, would also bear the same benefits. Our work showed that the thickness of the epidermis, however, was not reduced after treatment in the same manner as for the dermis. Blood vessels terminate in the dermis, while the epidermis is nourished via diffusion from there (Cevc & Vierl, 2007). This may account for the faster responses seen in the dermis.

In our preliminary clinical trial in the target species, dogs with naturally occurring atopic dermatitis also benefited from the use of oral nucleotides. There were improvements in pruritus and erythema, but alopecia and excoriation were not reverted by the treatment. This may be due to the short treatment course and study follow-up, since alopecia and excoriation are usually inflicted by the dog. A behavioral change, which can take longer, may be needed for the reduction of these lesions (Harvey et al., 2019).

Our study did not investigate mechanisms of action, and we can only speculate on this. The benefits seen here with the experimental treatment in mice (reducing dermal thickness and inflammation) and in the dog trial are possibly due to its capacity of interfering with inflammation via nucleotide receptors on immune cells (Chen et al., 2014). Polynucleotides activate the A2A adenosine receptor, which blocks intracellular pathways of inflammation, such as the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) cascades. Also, polynucleotides are expected to improve dermal repair through activation of the extracellular signal-regulated kinase (ERK) pathway in dermal fibroblasts, among other molecular mechanisms (Kim, 2025; Pitassi et al., 2024). Indeed, in a DNCB mouse model of atopic dermatitis there was an improvement in skin barrier and a reduction in several cytokines, including IL-4, IL-5, IL-13, IL-25, IL-33) (Ha et al., 2025). Therefore, A2A receptor activation can account for some of the clinical benefits of the experimental treatment used here. Nevertheless, A2A agonists also may drive IL-31 expression by TH2 cells, worsening pruritus and the clinical condition of atopic dermatitis (Kawano et al., 2023). This may respond as to why pruritus was not further reduced in our trial with dogs.

Manipulation of the nucleotide pool is a potent antimicrobial mechanism and can act through various other pathways, besides the A2A receptor. Other mechanisms include TLR, NLR and cGAS-like receptor activation (Hochhauser and Sorek, 2025). Many of these pathways may have been involved, although not measured here. This is one of the limitations of our study, in that we cannot ascertain which mechanisms were involved and which pathways could be further altered to improve clinical outcomes of the experimental treatment. Nevertheless, we

believe the work contributes in giving a first account on the possible benefits of nucleotide treatment in canine atopic dermatitis.

CONCLUSION

The reduction in pruritus can improve quality of life for dogs affected by atopic dermatitis and their guardians (Linek & Favrot, 2010). This study indicates that oral nucleotide administration may bear such benefits. Nevertheless, more studies are necessary to better understand the efficacy, dosage variations, mechanisms, and long-terms effects from this product.

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Conflict of interest statement.- All authors, except for Matheus C. Costa were also associated to Imunova at the time of project execution. Imunova has market positions in biotechnological therapeutics for atopic dermatitis.

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Data availability statement.- Data are available upon reasonable request to the corresponding author.

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Figures

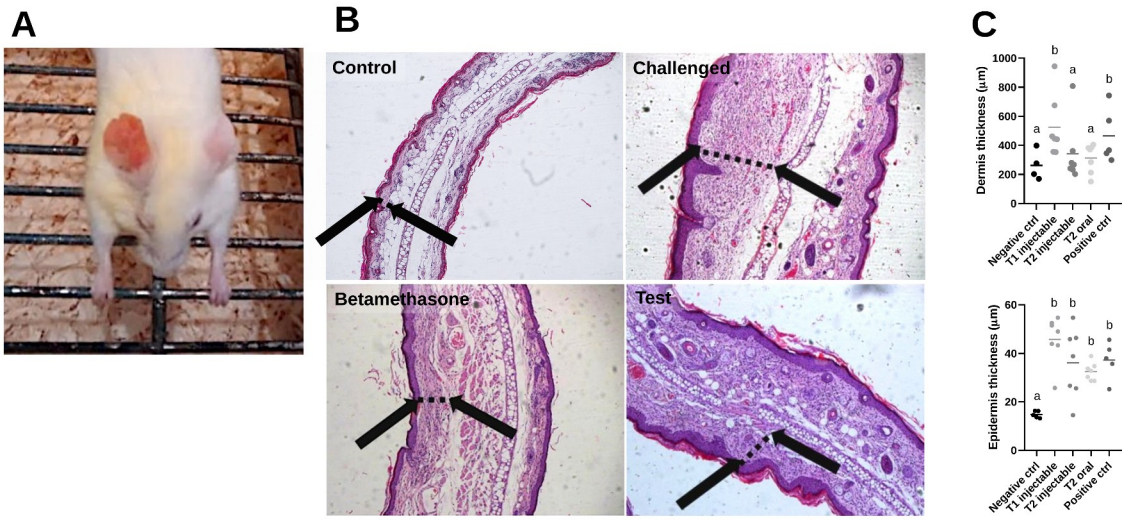

Fig. 1. - Induced atopic dermatitis (AD) and effect of nucleotides in mice. Experimental induction of AD lead to clinical signs that were compatible with the naturally occurring canine disease. The right ear showed reddening and sensitivity to the touch. The left ear was left unchallenged for comparison (A). Microscopic features of the induced AD. There was visible change in dermal and epidermal thickness. Representative results from the pilot and main mouse trials. The arrows indicate how these parameters were measured (10 measurements per ear section) (B). Dermis and epidermis thickness from the main mouse trial. Graphs represent the median ± 95% CI from 10 individual measurements of 3 ear sections from each mouse. Statistical analysis by unpaired t with Welch´s correction (dermis) or one-way ANOVA (epidermis). Statistical comparison was not possible in the pilot test due to low N. 10× magnification, HE stain (C).

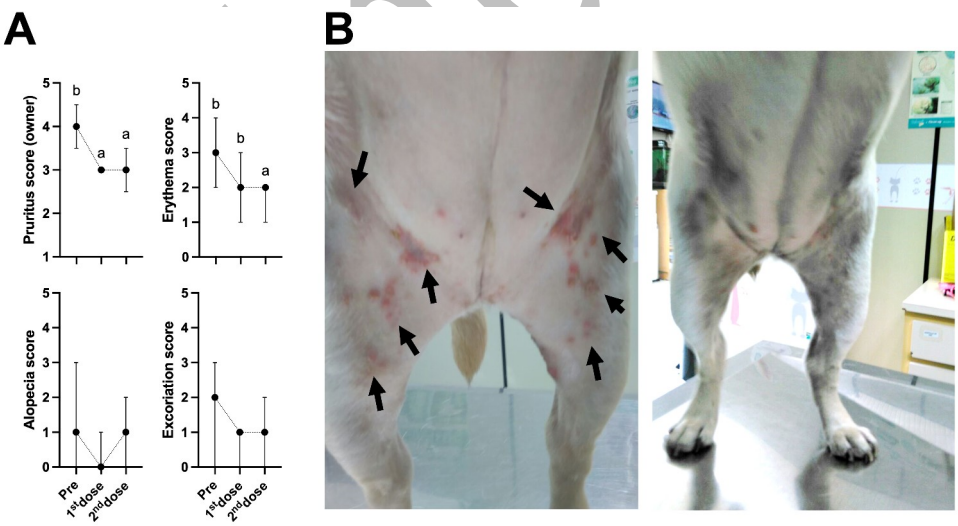

Fig. 2. - Clinical trial of nucleotides in dogs. Scoring of lesions (veterinarian assessment) or of pruritus (owner [guardian] assessment). Bars show the median and dots show individual dog scoring pre- or post-treatment (evaluations performed 1 week after each treatment). Statistical analysis by Kruskal-Wallis comparing all groups simultaneously (A). Representative clinical result of a dog treated with the test compound. Arrows on the left image show areas of intense erythema pre-treatment. The image to the right shows the same region by the end of the trial (B).

Table 1

Pilot trial				Main trial			
Group	Challenge (induced dermatitis)	Treatment †	N	*Group*	Challenge (induced dermatitis)	Treatment‡	N
Negative control	-	-	1	*Negative control*	-	-	5
Positive control	+	-	2	*Test 1 (T1)*	+	Nucleotides (20µg/animal) injectable	7
Betamethasone	+	Betamethasone (32 µg/animal)	2	*Test 2 (T2) injectable*	+	Nucleotides (20µg/animal) + $AlOH_3$ injectable	7
Test	+	Nucleotides (80µg/animal)	2	*Test 2 (T2) oral*	+	Nucleotides (20µg/animal) + $AlOH_3$ oral	7
				Positive control	+	-	5

N, number of animals in each experimental group.
†In the pilot mouse trial, the treatment was conducted weekly for 3 weeks following the induction of atopic dermatitis (on D35). Polynucleotides are a proprietary composition of Imunova Análises Biológicas LTDA.
‡In the second mouse trial, treatment was conducted on D18 and on D25 (before the end of the protocol for induction of atopic dermatitis, on D35