FACULTEIT DIERGENEESKUNDE
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INVITATION PUBLIC DEFENSE

SEARCHING THE RIGHT SPOT: ELISPOT AND SUBLINGUAL DROPS IN THE MANAGEMENT OF CANINE ALLERGIES

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PROMOTERS
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Curriculum Vitae

Michael Pelst was born on the 7th of May 1991 in Wilrijk, Belgium. In 2009, he obtained his diploma 'Mathematics-Sciences' at Sint-Ludgardisschool Merksem and commenced the studies of veterinary medicine at Ghent University in the same year. In 2015 he obtained the degree 'Master of Veterinary Medicine – Specialisation Research', having spent the final year conducting research for his master thesis which focused on the effects of peanut-specific sublingual immunotherapy on the immune response of healthy dogs. This thesis was awarded a prize for best master thesis of the specialisation research. Seeking to continue this research, he applied for and obtained an FWO strategic basic research grant which allowed him to start a PhD research project that focused on improving the management of allergic diseases in dogs.

Michael Pelst authored several scientific articles in peer-reviewed international journals and presented his research on multiple European allergy and immunology conferences.

How to attend?

The public defense will take place on **Tuesday the 13th of July at 17h30**, Auditorium Maximum (entrance 22), Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke and will be followed by a corona-proof reception.

Under the current COVID-19 restrictions, the defense can be attended in the auditorium by a limited audience (50 pers.) and will be broadcasted through live-stream.

In case you would like to be present at the public defense on campus or you would like to attend the defense through live-stream, please register before the Wednesday the 7th of July by email: Michael.Pelst@UGent.be. A link to the online presentation will be sent.

Members of the Examination Committee

Prof. Dr. H. De Rooster
Chair of the examination committee,
Faculty of Veterinary Medicine, Ghent University

Prof. Dr. F. Broere
Faculty of Veterinary Medicine, Utrecht University

Prof. Dr. D. Bullens
Faculty of Medicine, KU Leuven

Prof. Dr. P. Gevaert
Faculty of Medicine and Health Sciences,
Ghent University

Dr. S. Vandenabeele
Faculty of Veterinary Medicine,
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Summary of the thesis

The management of allergic skin diseases in dogs often forms a nuisance to veterinarians. No laboratory method is currently reliable enough to diagnose the allergens which cause the disease. Additionally, although symptomatic treatments control the illness in most dogs, these therapies do not deal with the underlying problem, a dysregulated immune system. Allergen-specific immunotherapy is the only therapy that can alter the course of the immune response. However, due to its moderate success rate, this therapy is not a first choice treatment in general practice. To tackle these problems, this thesis evaluated whether a novel approach could diagnose the causative allergens in dogs with skin allergies. Furthermore, by studying canine oral epithelial cells, ways to improve the efficacy of allergen-specific sublingual immunotherapy were investigated.

Chapter I's literature overview provides a background on the established scientific knowledge relevant to this thesis. First, information is given on some of the cells and immunological mediators which are involved in skin allergies. Second, the usefulness of IgE allergy tests in dogs with skin allergies is discussed. Third, emphasis is put on the different therapies which can be used in dogs with an allergic dermatitis, disclosing the most commonly used symptomatic treatments and giving more in depth information on the efficacy and immune effects of allergen-specific immunotherapy. Fourth, an overview is given on the most common allergen sources in dogs. Fifth, the finding that these allergens can trigger innate immune responses is discussed. Sixth, the ability of allergens to trigger immune mediator secretion by epithelial cells is deepened. Seventh and final, knowledge is provided on the characteristics of the cells which line the oral mucosa, the oral epithelial cells.

In **Chapter II**, an overview of the aims of the PhD thesis is given.

Chapter III investigated whether the use of peanut-specific sublingual immunotherapy allows the study of how allergen-specific cellular and humoral immune responses are generated in healthy dogs. Of the four dogs in the treatment group, only one animal had a distinct humoral immune response. Even though this dog showed an increase in peanut-specific IgE titers, no circulating peanut-specific IgE-secreting cells could be detected using the ELISPOT technique. Still, it was shown that the therapy has the potential to induce peanut-specific antibody responses in the blood plasma, saliva and peripheral blood mononuclear cells of a healthy dog.

Chapter IV investigated the diagnostic value of using ELISPOT to detect circulating allergen-specific antibody-secreting cells in dogs suffering from clinical skin allergies. While no allergen-specific IgE-secreting cells could be detected, allergen-specific IgA- and IgG-secreting cells were observed for several common allergens. Still, no significant difference was observed in the frequencies of these cells between healthy and allergic animals. Therefore this study did not obtain any evidence that ELISPOT can be used as an aid to diagnose skin allergies in dogs. Nevertheless, this chapter's methodological approach allows the study of circulating antibody-secreting cells in dogs.

When sublingual immunotherapy is given, allergen extracts are applied under the tongue to desensitise the patient for this specific allergen. Because oral epithelial cells are the first cells which come in contact with the allergen, **Chapter V** assayed how allergens and potential adjuvants can impact mediator secretion by canine oral epithelial cells. Studying an immortalised cell line of canine buccal epithelial cells, it was shown that these cells only produced a limited number of pro-

inflammatory cytokines. CXCL8 secretion could be augmented by a *Dermatophagoides farinae* extract, an *Alternaria alternata* extract, Toll-like receptor 2 ligands (Pam3CSK4, heat-killed Listeria monocytogenes and FSL-1), the Toll-like receptor 5 ligand flagellin and canine recombinant IL-17A. Calcitriol, the most active form of vitamin D3, on the other hand significantly suppressed CXCL8 secretion by the immortalised cells. The obtained knowledge on these interactions provides an aid in determining how the generation of tolerance can be impacted at the oral epithelial cell level.

In **Chapter VI**, we investigated whether the findings of Chapter V were reproducible for primary canine sublingual epithelial cells. Indeed, also the sublingual cells showed limited variety in pro-inflammatory cytokine production. The cells responded with an increased CXCL8 production to the *Dermatophagoides farinae* extract and Toll-like receptor 2 ligands while calcitriol suppressed the secretion of this chemokine. Additionally, RNA-sequencing was performed to obtain improved insights on the expression profile of these oral epithelial cells. This revealed that *in vitro* cultured canine sublingual epithelial cells do not express the pro-allergic mediators TSLP, IL-25 and IL-33, unlike what has been reported for other types of epithelial cells. Sublingual epithelial cells therefore seem to possess some inherent anti-inflammatory and anti-allergic properties which likely facilitates the generation of tolerance.

Chapter VII provides a deeper discussion on potential approaches to improve allergy diagnosis in dogs. Furthermore, information is given on strategies to enhance the efficacy of sublingual immunotherapy. Moreover, the findings on the anti-inflammatory and anti-allergic properties of the isolated oral epithelial cells are highlighted. Ultimately, certain overarching considerations and limitations of the conducted studies are discussed.