Relatório sobre Proposta de Unidade Curricular de Imunoalergologia Médico-Veterinária

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Report on a Proposal for a Curricular Unit in Veterinary Immunoallergology

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i. List of abbreviations

ASIT – Allergen-specific Immunotherapy.

BALF – Bronchoalveolar Lavage Fluid.

BAT – Basophil Activation Test.

cAC – Canine Allergic Conjunctivitis.

cAD – Canine Atopic Dermatitis.

CAST – Cellular Antigen Stimulation Test.

CCD – Cross-reactive Carbohydrate Determinants.

CD – Cluster of Differentiation.

CRD – Component-resolved Diagnosis.

CRIT – Component-resolved Immunotherapy.

DC – Dendritic Cells.

ECP – Eosinophil Cationic Protein.

ECTS – European Credit Transfer System.

ELISA – Enzyme-linked Immunosorbent Assay.

EPIT – Epicutaneous Immunotherapy.

fAS – Feline Atopic Syndrome.

FCƐRI – High affinity receptor for immunoglobulin E.

FCT – The Portuguese Foundation for Science and Technology.

IAD – Inflammatory Airway Disease

IBH – Insect Bite Hypersensitivity.

ICADA – The International Committee on Allergic Diseases of Animals.

IDT – Intradermal Tests/testing.

IEF – Isoelectric Focusing.

IFN-γ – Interferon gamma.

Ig – Immunoglobulin.

IL – Interleukin.

ILIT – Intralymphatic Immunotherapy.

IUIS – International Union of Immunological Societies.

LTT – Lymphoblastic Transformation Test.

MADx – Macroarray Diagnostics.

MOODLE – Modular Object-Oriented Dynamic Learning Environment.

mRNA – Messenger Ribonucleic Acid.

NFHD – Non-flea-induced Hypersensitivity Dermatitis.

OFC – Oral Food Challenge(s).

PAX – Pet allergy Xplorer.

PBMCs – Peripheral Blood Mononuclear Cells.

RAO – Recurrent Airway Obstruction

RAST – Radioallergosorbent-test.

SCIT – Subcutaneous Immunotherapy.

SDS-PAGE – Sodium Dodecyl-sulphate Polyacrylamide Gel Electrophoresis.

SEA – Syndrome of Equine Asthma

SIIUE – "Sistema de Informação Integrado da U. Évora" (Integrated Information Service of the University of Évora).

SLE – Lupus Erythematosus.

SLIT – Sublingual Immunotherapy.

sLT – Sulfidoleukotriene.

SPAIC – "Sociedade Portuguesa de Alergologia e Imunologia Clínica" (The Portuguese Society of Allergology and Clinical Immunology).

TGF-β – Transforming Growth Factor-beta.

Th – T helper cell(s).

Treg(s) – T regulatory cell(s).

1. Preamble: Veterinary immunoallergology. How it started and evolved, and the motivation for the matter

Allergy is currently considered the major non-infectious disease as it has gained epidemic proportions over the last few decades. In fact, despite being known, allergic diseases were practically "non-existent" or at least remained on stand-by until 200 years ago.

The first scientific description of allergy in humans probably occurred in the 19th century, with experimental studies showing the elicitor role of pollens (Ring 2022). The discovery of immunoglobulin (Ig) E in 1967 allowed for the understanding of the pathophysiology of anaphylaxis and paved the way for immunotherapy (Bergmann and Ring 2014).

Already in the 20th century, remarkable scientific knowledge evolved in the field of immunology and allergy, first with the Prausnitz-Küstner test, and then, after the discovery of IgE, with the development of the Radio-Allergo-Sorbent-Test (RAST), for the routine detection of allergen-specific IgE (sIgE). By describing T helper cells (Th) 1 and Th2 subsets in immune cell studies as well as mast cells and basophils, further knowledge was obtained from histamine release studies. Then, leukotrienes were discovered, and all this allowed the development of pharmacotherapy in the early decades of the 20th century, with epinephrine, antihistamines, and cortisone (Ring 2022).

As in humans, veterinary immunoallergology covers hypersensitive conditions triggered by allergens, affecting different organs such as skin, digestive tract, and bronchial tree, in several species, from which dogs, cats and horses are the most studied. Focusing on the immune system of animals, it studies the mechanisms associated with the development of allergic conditions, identifying the triggering factors, aiming to control clinical manifestations.

As a veterinarian, and already teaching in the field of veterinary medicine, upon the conclusion of the PhD in 2007, the previous research in human allergy was about to shift to the homologous veterinary domain.

The PhD research had allowed me to get in close contact with different methods. It was possible to train and perform electrophoretic techniques such as isoelectric focusing (IEF) and polyacrylamide gel electrophoresis (PAGE), western-blotting and its inhibition, as well as radioallergosorbent-test (RAST) inhibition. These methods allowed us to perform relevant allergen separation and identification of patient sensitization, and were followed by the evaluation of immune cell response upon contact with molecular allergens, through the *in vitro* provocation of peripheral blood mononuclear cells (PBMCs), subsequently analyzed through immunophenotyping detection of produced interleukins, in flow cytometry. Then, the application of these methodologies to studies on animal allergy was a real challenge to come.

If several training courses had helped me to better understand those methods, and short-time intensive internships in units like the "L'Unité Immuno-Allergie de l'Institut Pasteur" (Paris) or the Clinical Immunology Laboratory at Charité Campus Mitte (Berlin) allowing to acquire the essential skills that would be used in related research, the shift to veterinary immunoallergology, besides less species-specific reagents available, demanded funding that soon have showed unavailable.

As a Portuguese, we are used to the proverb "a necessidade aguça o engenho", which English equivalent is probably "necessity is the mother of invention", and if we know where we want to go, we make the path as we go. As equipment and reagents were needed, I started to search for it within the university as well as in friendly institutions. At the end, in 2008, the essential equipment was already available, and after an unsuccessful application for a public post-Doc scholarship, a second application was well succeeded, providing a small yet essential funding.

The post-Doc plan "Allergological characterization for *Dermatophagoides pteronyssinus* (mite) and *Dactylis glomerata* (grass) of canine populations from the regions of Évora and Lugo, Spain" has been approved and would run from 2009 to 2012 as the first large study in veterinary allergy in Portugal.

Nevertheless, it was necessary to start collecting and characterizing clinical cases, and in a small city as Évora it was another real challenge. However, as dermatological issues were already increasing at the time, a few dozens of frameable patients attending the university hospital outpatient consultation were added to the study, along with another group from the dermatology outpatient consultation from Rof Codina University Hospital (Lugo, Spain), firstly characterized by Prof. Ana Goicoa.

With a small monthly bench scholarship, essential reagents were progressively acquired, and the work proceeded with the disclose and publication of the allergomes from three relevant allergen species (*Dermatophagoides pteronyssinus*, *Dactylis glomerata* and *Phleum pratense*) for dogs.

That kind of work is especially important to disclose different species' allergomes for animals as it is more extensively characterized for humans, and the relevance of some allergens is shared between humans and animals. Furthermore, inhibition techniques may also allow the identification of cross-reactivity phenomena, a relevant issue when equating the more adequate allergen pool for immunotherapy. These techniques are currently being used in the extensive adaptation for veterinary allergy of cutting-edge microarray diagnostic methods, such as Pet allergy Xplorer (PAX®) from Nextmune (Stockholm, Sweden) and I feel proud to cooperate with.

Along with the development of new drugs, which together with more traditional ones, may allow improving animal allergy therapeutic approach, a more precise diagnosis in terms of identification of the implicated allergenic species is essential to perform effective avoidance measures and equate allergen-specific immunotherapy (ASIT). However, as animals are not allergic to a species itself, but to the allergens from it, obtaining a more effective ASIT clinical result will demand a more refined molecular diagnosis, identifying cross-reactive determinants as well. This high precision diagnosis/immunotherapy combined focus resulted in what became known as Component-resolved Diagnosis (CRD) and Component-resolved

Immunotherapy (CRIT), falling into the context of precision medicine. However, it demands deeper diagnostic investigation and adaptation through modern diagnostic methods.

Shortly after beginning my post-doctoral research in 2010, I organized the first Diagnostic Update in Veterinary Dermatology and Allergology course (*Jornadas de Atualização Diagnóstica em Dermatologia e Alergologia Veterinárias*), held in Évora from April 30 to May 2. The course was integrated into the pedagogical component of the Medical Semiology II discipline, specifically the module on dermatological diagnosis, and was also open to the wider veterinary community. It was credited with 1 European Credit Transfer System (ECTS) and received partial funding from the *Fundo de Apoio à Comunidade Científica* of the Portuguese Foundation for Science and Technology (FCT).

Meanwhile, a few hundred animals have been observed in the university immunoallergology outpatient consultation, which was officially implemented in 2016. This number of patients was only possible with the cooperation of veterinary colleagues, mostly from Évora surrounding areas, promoted by the word spreading between the owners.

Participation as an associate of the European Academy of Allergy and Clinical Immunology (Interest Group of Comparative and Veterinary Allergy) and as co-founder and first coordinator (2021-2023) of the "Grupo de Interesse de Imunoalergologia Comparada e Veterinária" from the "Sociedade Portuguesa de Alergologia e Imunologia Clínica" (SPAIC) enhanced the opportunities as well as the responsibilities. Then, inserted in an intense activity for veterinary allergy promotion, the opportunity to coordinate a first edition of the Advanced Training in Veterinary Immunoallergology arose, and the course occurred from March to May 2023, conferring 2 ECTS to the participants. This first b-learning edition caught the attention of the veterinary class, and 16 professionals successfully completed the formation. After that, other colleagues got in contact, asking for another edition, which in addition to the increasing clinical expression,

plus the evolution of diagnostic possibilities, suggested the interest in creating a new optional curricular unit, specifically directed to the veterinary training in this subject of increasing clinical relevance.

2. General scope and objectives

Training in veterinary immunoallergology will focus on basic immunology, starting by studying the physiology of the immune system and its main role of protection against pathogens. It is followed by a review of the anatomy and physiology of the main target systems such as skin, respiratory and digestive tracts.

By enabling the use of basic concepts of immunology related to sensitization and allergy, as well as dermatology, pneumology and gastroenterology, trainees will develop scientific query skills and clinical examination, and prescribe complementary diagnostic tests in order to produce a rational diagnosis through evidence-based reasoning.

The curriculum content of this discipline will better fall in the context of the fifth year of the Integrated Masters's in Veterinary Medicine of the University of Évora, especially in the second semester, integrating the Option 3 group of curricular units. In the fifth year of formation, students have already studied matters from biochemistry, physiology, immunology, and medical semiotics, with marked relevance for the understanding of the contents covered here. Simultaneously, companion animal and equine medicine are developed during the fifth year of the course, contributing to an integrated approach.

In addition to fifth-year students of the Integrated Masters's in Veterinary Medicine, this optional curricular unit should be also directed to veterinary professionals, and health professionals in general, in the context of lifelong learning and One Health. The b-learning format, in English, will also allow the proficiency of this objective, facilitating the recruitment of international students. The maximum number of students should be 50.

The **specific objectives** of a Veterinary Immunoallergology curricular unit are the acquisition of the following skills:

i) search for updated scientific and technical information;

ii) understand the pathophysiology of the involved mechanisms;

iii) interpretate the results of each diagnostic method;

iv) understand the possibilities of each therapeutic option in the context of a multimodal approach;

v) assess clinical contexts for diagnosis formulation, using differential plans for different conditions;

vi) produce a positive diagnosis as complete as possible;

vii) understand and explain to third parties the pathophysiology mechanisms of each process under analysis, equating available prophylactic and therapeutic approach for each case;

viii) present and discuss the most recommended therapeutic approach for each case;

ix) present a prognosis, justifying the proposed timetable for successive monitoring, and

x) successively re-evaluation of the allergic patient, allowing diagnosis adjustment and consequent prophylaxis and treatment reformulation.

3. Workload and teaching methodology

The curricular unit on Veterinary immunoallergology is planned for an academic semester, with the following class load:

- Contact hours – one weekly theoretical-practical class of 3h (3h/week; weeks 1 to 13) and one week tutorial class of 2h (week 14);
- total duration of the unit – 14 weeks;
- total contact – 41h;
- total dedication time – 78h (3 ECTS).

The program will be made available through the academic portal of the University of Évora – "Sistema de Informação Integrado da U. Évora" (SIIUE) and Modular Object-Oriented Dynamic Learning Environment (MOODLE).

The **theoretical-practical classes** will be composed of lectures based on PowerPoint presentations containing the main concepts, with short texts, figures and pictures, integrating the essential knowledge on each programmatic subject. A few sample slides are available ahead.

Critical thinking-oriented study will be encouraged, based on objective evidence-based reasoning, starting with problem identification, supported analysis for rational interpretation, aiming at an evaluation, allowing logical conclusions based on scientific arguments.

Based on the theoretical lectures, students will deepen their understanding of immunoallergology by studying mainly the essential references, allowing the acquisition of further skills when discussing scientific articles in journal clubs. The critical thinking stimulation will proceed through diagnostic and treatment reasoning of different clinical cases.

Different clinical conditions needing diagnostic reasoning for differential diagnosis will then be presented. Some of them will be related to different immune-mediated diseases and others not. Evidence-based interpretation will be the focus of the discussion, aiming at the establishment of a positive and as far as possible complete diagnosis.

The approach to the given matters will be supported on revision texts, either textbooks or papers, and on relevant original manuscripts, which will be indicated/made available through the MOODLE platform.

Student-triggered questions and discussions of presented topics are welcomed during the lectures, stimulating a proactive attitude and increasing interest regarding the subjects addressed. In-line with an interactive attitude, lecturing matters will be subject to questions by the teaching staff, to stimulate the reasoning of the students regarding the information they are receiving and based on the knowledge they already

have. In another way, upon a given diagnosis, the treatment options will be equated and discussed according to the objectives (*e.g.* healing, just clinical sign relieving or palliative) and limitations (*e.g.* medicine availability, comorbidities, administration friendliness or even cost).

The easiness/difficulty observed through the reasoning processes by the students will be evaluated according to the possible need for additional revision through the basal knowledge.

At the end of each class, the main concepts and key points will be outlined, related to other relevant concepts and to the next matter, when indicated.

Under the theoretical-practical way, different matters will be addressed. Some of them will be studied in a less hands-on way, like those related to the in-depth approach on hypersensitivity concepts and mechanisms, identification and characterization of allergens, or immune cell assessment, and others by hands-on methods, like skin cytology, trichoscopy or intradermal testing. However, both mainly clinical discussion and hands-on class ways will fall into the theoretical-practical model as an introductory conceptualization will start the lecture.

The first and introductory part of the lecture will be made for all the group of students. Then, according to the novelty/complexity of the matter, subsequent discussion may follow the same organization or be subject of previous preparation by subgroups of 6-8 students, in subthemes, which will be subsequently presented, followed by discussion. For instance, after the conceptualization of atopic dermatitis, the symptomatology of a given clinical case may be subjected to diagnostic and treatment approach by different subgroups of students: subgroup 1 will address differential diagnosis regarding other possible skin conditions, subgroup 2 will address the identification of the implicated allergen species through intradermal testing, subgroup 3 will address the sensitization through serum testing, subgroup 4 will address the possibilities for treatment approach in terms of allergen avoidance, pruritus and infection control, and subgroup 5, the skin barrier improvement and allergen-specific immunotherapy options.

The complementary diagnostic methods in immunoallergology, either *in vivo* or *in vitro*, will be also addressed. A previous lecture will take place, explaining the fundamentals and then the results regarding different clinical cases are presented and discussed as bridges between the clinical diagnosis and the rational for treatment implementation. Regarding *in vivo* methods like intradermal testing, further hands-on practice will take place with clinical cases from the University Veterinary Hospital or associated Veterinary Centres outpatient consultation.

Finally, different extensively documented clinical cases will be object of discussion.

There will be classes based on lectures, discussion and fundamentals studying, and others adopting the Journal Club format, discussing the fundamentals' chronological evolution and state-of-the-art findings in the field of veterinary immunoallergology. Students will be encouraged to present clinical cases based on their clinical activities, followed by discussion.

The last class will take the tutorial format, aiming to summarize matter-related objectives, coaching the students for independent search for information on reliable sources as a complement for the acquired knowledge and reasoning skills, thereby stimulating the critical thinking they will lifelong need for a fruitful self-learning process. This teaching-learning approach will stimulate the understanding capacity, instead of memorizing, which will favor the ability to apply the previous knowledge to new situations.

At the end, a written evaluation will take place, covering all the matter, composed of the following possible question format: direct short answering, multiple choice and essay questions. Answering may be question-derived directly or result from given clinical cases interpretation.

4. The curricular unit on Veterinary Immunoallergology

4.1. Program

The list of the matters addressed during the 14 weeks of the curriculum is as follows.

Week/Class 1

Introduction to the Curricular Unit.

The immune system – constitution and action.

Mechanisms of hypersensitivity and allergy.

Week/Class 2

Atopy – Evolution of concepts up to the current state of the art.

Week/Class 3

Diagnosis of atopy in dogs.

Journal club 1: discussion of papers on diagnosis of the phenotypes of atopy in dogs.

Week/Class 4

Diagnosis of atopy in cats and horses.

Week/Class 5

Feline and equine asthma:

- Diagnosis;

- Journal club 2: discussion of papers on the pathophysiology of asthma.

Week/Class 6

Ocular allergy.

Food allergy.

Week/Class 7

Allergy to insect bites in horses.

Contact allergy.

Week/Class 8

Identifying and characterizing allergens.

Week/Class 9

Complementary methods in allergy diagnosis – Clinical and laboratorial methods.

Week/Class 10

Complementary methods in allergy diagnosis – Intradermal testing.

Week/Class 11

Therapeutic approach to allergy in dogs, cats and horses.

Journal club 3: addressing the skin barrier.

Week/Class 12

The allergen-specific immunotherapy and the multimodal treatment approach.

Week/Class 13

Presentation and discussion of clinical cases by the students, based on their clinical activities – I.

Week/Class 14

Presentation and discussion of clinical cases by the students, based on their clinical activities – II.

Summary of the matter-related objectives and student coaching.

4.2. **Theoretical-Practical Classes**

Class 1

 # Introduction to the Curricular Unit

 # The immune system – constitution and action

 # Mechanisms of hypersensitivity and allergy

A **general introduction to the Curricular Unit** will be done in terms of i) the topics covered; ii) how they are framed in the Integrated Master´s in Veterinary Medicine; iii) teaching methodology (theoretical-practical classes and how they are developed), and iv) the evaluation methodology.

A **brief history of allergology** will be presented, exposing the evolution of knowledge in the field, since the episode of pharaoh Menes being stung by a wasp and dying, 2600 BC, from what we currently understand as anaphylaxis (Krombach et al. 2004).

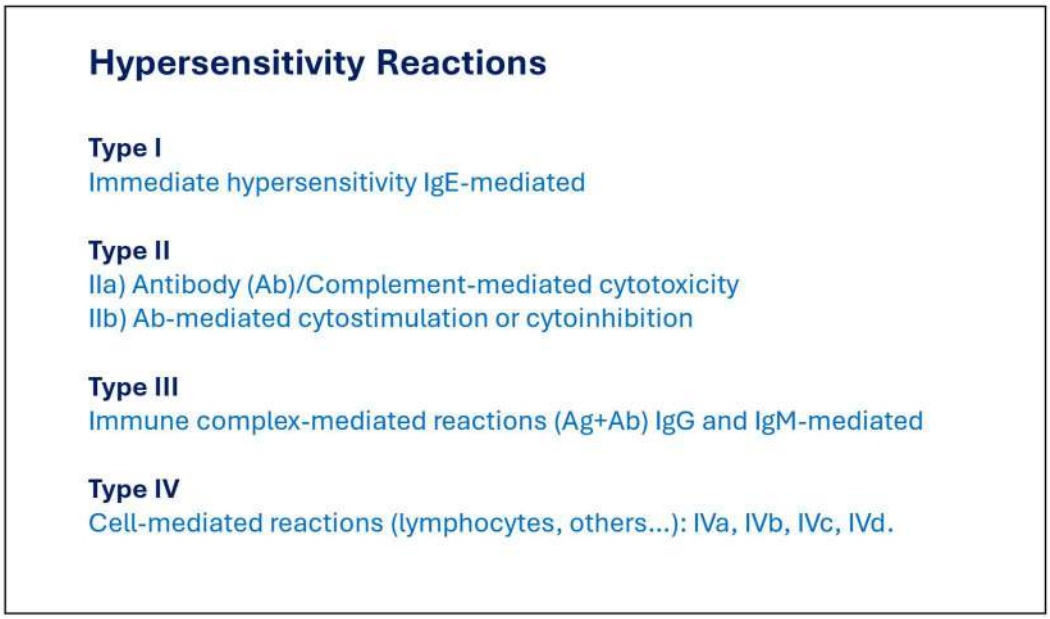
In this class, the lecture focuses on **reviewing the immune system**, its main components as the lymphoid organs (bone marrow, thymus, spleen and lymph nodes) and leucocytes. Their main interactions will be addressed by cytokine, antibody and complement functions. Innate and adaptative immunity, acting through cell mediation and humoral action, will be addressed.

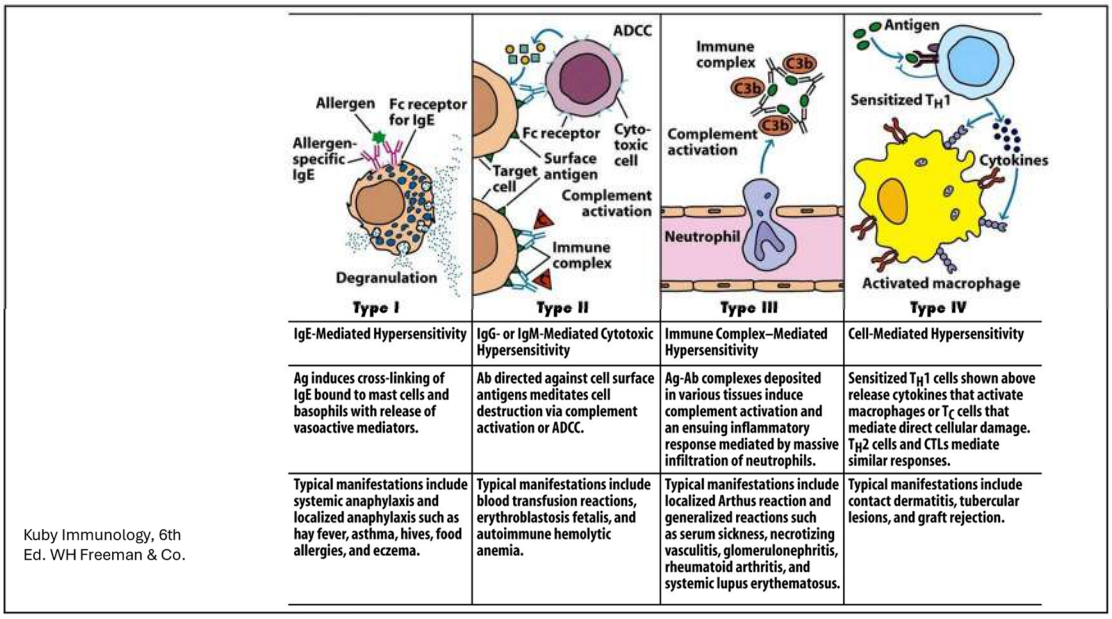
Main system interactions will be addressed through cytokine, antibody and complement functions, regarding hypersensitivity, to promote the understanding of the physiopathogeny of allergic conditions.

Based on the immune functions, the hypersensitivity reactions will be addressed as they play a key role in allergy. The **four types of hypersensitivity** are then presented as: i) **Type I (immediate) hypersensitivity**, which is mediated by IgE fixed on mast cells, binding to antigens (allergens) and triggering mast cells to release mainly histamine, originating several allergic conditions as atopic dermatitis,

asthma or different forms of food allergy; ii) **Type II antibody-mediated cytotoxic hypersensitivity**, where IgG/IgM target cells, leading to their impairment, like in immune-mediated hemolytic anemia or transfusion reactions; iii) **Type III immune complex-mediated hypersensitivity**, where immune complexes (antigen-antibody) deposit in tissues and activate complement, leading to inflammation, like in systemic lupus erythematosus (SLE), possibly causing glomerulonephritis, arthritis, dermatitis and vasculitis, or the so called blue-eye in viral hepatitis or upon its vaccination, and iv) **Type IV delayed-type (cell-mediated) hypersensitivity**, where T-cells mediate the immune response, like in contact dermatitis, patch allergy testing or tuberculin skin tests.

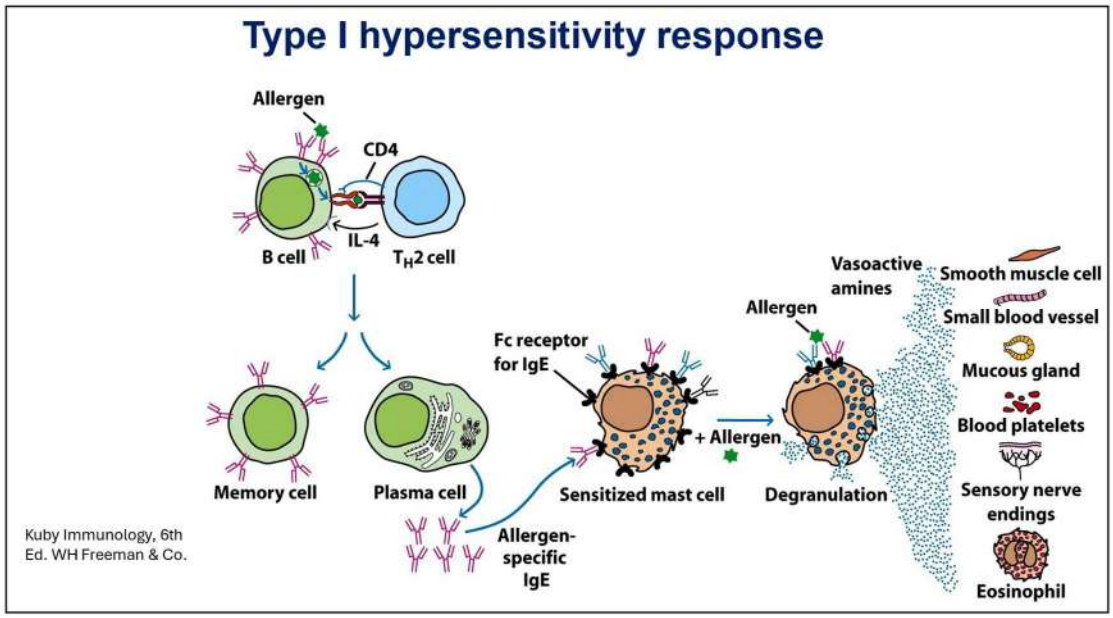
Examples of slides to be used in Lecture 1:





Type I	Type II	Type III	Type IV
IgE-Mediated Hypersensitivity	IgG- or IgM-Mediated Cytotoxic Hypersensitivity	Immune Complex–Mediated Hypersensitivity	Cell-Mediated Hypersensitivity
Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.	Ab directed against cell surface antigens meditates cell destruction via complement activation or ADCC.	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils.	Sensitized T_H1 cells shown above release cytokines that activate macrophages or T_C cells that mediate direct cellular damage. T_H2 cells and CTLs mediate similar responses.
Typical manifestations include systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema.	Typical manifestations include blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia.	Typical manifestations include localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus.	Typical manifestations include contact dermatitis, tubercular lesions, and graft rejection.

Kuby Immunology, 6th Ed. WH Freeman & Co.



Class 2

 # Atopy – Evolution of concepts up to the current state of the art

This class integrates a lecture explaining the **concept of atopy** in its evolution and current state of diagnostic identification in different species.

In veterinary medicine, the first rules for atopy diagnosis, including two sets of criteria (one major and one minor) were formulated in the 1980s,

regarding canine atopic skin disease (Willemse 1986). Then, Prélaud et al. (1998) proposed two new sets of criteria (again, a major one and a minor one) for the diagnosis of canine atopic dermatitis (cAD) in an itchy dog, after the exclusion of ectoparasite-related causes. It would allow a diagnosis of atopy with roughly 80% sensitivity and specificity. Then, Počta & Svoboda (2007) assessed the criteria previously proposed by Willemse (1986) and by Prélaud et al. (1998), and established their sensitivity in, respectively 72.3% and 68.1%, with no statistically significant differences between them.

Later, Favrot et al. (2010) reported the results of a prospective study comparing 843 dogs from different continents, diagnosed with atopic dermatitis, with a second group of dogs with other pruritic disorders. The sensitivity and specificity of the criteria from Willemse and Prélaud were applied and compared the expression of those criteria in dogs with atopic dermatitis, whether induced by food or not. When tested in that vast international canine population, Willemse and Prélaud's criteria showed a substantial decrease in sensitivity/specificity. Significant differences were also found between animals where clinical signs were induced or not by food. Based on these observations, a new set of eight criteria was proposed, with a combination of five of these criteria leading to 85% sensitivity and 79% specificity. By increasing the requirements to the presentation of six criteria, specificity would rise to 89%, but sensitivity would decrease to 58%.

Addressing atopy will allow a better understanding of the diagnosis of most allergic conditions. It will focus on the former definition of atopy, proposed by Halliwell (2006) as "A genetically-predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is most commonly associated with immunoglobulin (Ig)E antibodies to environmental allergens", which has been the accepted definition for many years, and then on the new one, dated from 2024. Based on articles and meeting abstracts from 2015 to 2022, the International Committee

on Allergic Diseases of Animals (ICADA), concluded that an updated definition of cAD was needed, based on the most recent knowledge of its pathogenesis. Based on that, cAD was redefined as “A hereditary, typically pruritic and predominantly T-cell driven inflammatory skin disease involving interplay between skin barrier abnormalities, allergen sensitization and microbial dysbiosis” (Eisenschenk et al. 2024). Atopic dermatitis of animals could still be considered a genetically predisposed condition, with defined clinical patterns associated with IgE directed against environmental allergens as happens in humans (Bradley et al. 2023, Paterson 2023, Halliwell 2021, Favrot 2016, Favrot et al. 2012, Favrot et al. 2010), but the new definition could now cover the current broader understanding of the pathogenesis of cAD, which is known for extending beyond allergy. According to Eisenschenk et al. (2024) “this new definition highlights the multiple factors involved in this disease, which supports the recommendation that the management of canine atopic dermatitis must be multimodal”, as already considered by Olivry et al. (2015). Furthermore, this new and more comprehensive definition has shown more suitable to cover those similar clinical patterns, observed in cases where it is not possible to demonstrate the implication of IgE, either by intradermal or serological tests, which have been leading to the diagnosis of atopic-like dermatitis (Martins et al. 2016, Halliwell 2006).

By the end of the class, students will be challenged to summarize the concept of atopy, having in mind what should be present and what should be discarded for a positive diagnosis.

Examples of slides to be used in Lecture 2:

Prélaud's Major Criteria for Canine Atopy

1. Appearance of pruritus between the ages of 6 months and 3 years
2. Corticosteroid-sensitive pruritus
3. Bilateral cranial erythematous pododermatitis in limbs
4. Erythema of internal pinnae
5. Peribucal erythema/cheilitis

Prélaud's Minor Criteria for Canine Atopy

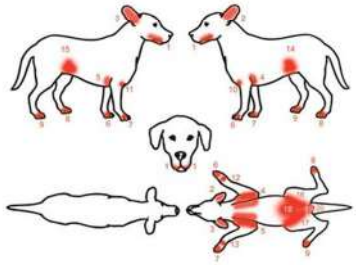
1. Predisposed breed or family background
2. Chronic or recidivant dermatitis for more than 2 years
3. Dull coat
4. Elbow wrinkle affection
5. Lick dermatitis
6. Hyperidrosis
7. History of hives or angioedema
8. Seasonal worsening
9. Worsening on the grass
10. Changing of symptoms according to the place

Favrot ´s Diagnostic Criteria for Canine Atopic Dermatitis

1. Onset of signs under 3 years of age
2. Dog living mostly indoors
3. Glucocorticoid-responsive pruritus
4. Pruritus *sine materia* at onset (i.e. pruritus without lesions at onset)
5. Affected front feet
6. Affected ear pinnae
7. Non-affected ear margins
8. Non-affected dorso-lumbar area

5-criteria → sensitivity ≈ 85%; specificity ≈ 79%

6-criteria → sensitivity ≈ 58%; specificity ≈ 89%



Favrot *et al* (2010)

Class 3

 # Diagnosis of atopy in dogs

 # Journal club 1: discussion of papers on diagnosis of the phenotypes of atopy in dogs

The lecture will focus on diagnosing atopy in dogs, starting with a proper workup, where other possible conditions will be discarded. A confirmative clinical diagnosis will be followed by complementary means to identify the implicated allergen sources.

The evolution of cAD diagnosis has been already addressed in class 2. Hence, the diagnosis of cAD will be performed by using the Favrot's set of criteria. By applying of Favrot's criteria, the International Task Force on cAD aimed at two main objectives: 1. as a diagnosis aid, and 2. to increase the homogeneity of the inclusion criteria of canine individuals in scientific studies. However, the simple application of these criteria still led to an appreciable diagnostic limitation, allowing for one misdiagnosis in every five cases, that is, a sensitivity and specificity rate of 80%. However, by tracking and discarding ectoparasites and skin infections, diagnostic specificity rate could show substantial increase (Olivry, 2010).

On the other hand, if one more criterion (six, instead of five) was demanded for a positive diagnosis, specificity would rise to 89%, increasing the precision of the diagnosis of atopy, but sensitivity would go down to 58%, resulting in further underdiagnosis (Olivry, 2010). Diagnostic limitations could somehow be minimized by further diagnostic methods such as intradermal testing (IDT) and serological determination of allergen-specific IgE, highlighting the relevance of identifying IgE-mediated reactivity, when considering allergen specific immunotherapy as an option (Olivry et al. 2010).

Several clinical cases will be subjected to this diagnostic approach, bearing in mind a combination of five or six of these criteria.

At the end of the class, a correct and extensive diagnostic approach to canine atopy will be summarized by highlighting requirements like the exclusion of: 1. ectoparasite infestations and infections, 2. endocrine or autoimmune diseases, 3. possible food allergy, and 4. allergy to flea bites. Then, the administration of the Favrot's criteria, and the identification of the relevant allergen species to which the individual is sensitized to, will be the focus.

In the second part of the class, in a **journal club** format, students will present and discuss 1-2 papers on phenotypes of atopy in dogs (*e.g.* Hensel et al. 2015).

Examples of slides to be used in Lecture 3:

Diagnosis of cAD

Identify other skin conditions with similar clinical signs (work-up)

Detailed interpretation of presentation and development (application of the Favrot's criteria)

Assessment of skin reactivity through IDT and determination of serum specific IgE (allergy tests)

Hensel *et al* (2015)
Martins *et al* (2016)

Aproved by the ICADA

Class 4

 # Diagnosis of atopy in cats and horses

The lecture on **atopic syndrome in cats** will be addressed also having in mind the need for a previous work-up in pruritic cats, to exclude possible flea hypersensitivity dermatitis, fungal and bacterial diseases, as well as some neoplastic and viral conditions (Favrot et al. 2012). Then, the diagnosis should rely on the identification of feline non-flea-induced hypersensitivity dermatitis (NFHD) (Favrot et al. 2012) considering that affected cats will frequently present with one of the following patterns: 1. head and neck excoriations, 2. symmetric self-induced alopecia, 3. eosinophilic skin lesions, and 4. miliary dermatitis. Two sets of criteria, one generic, comprising eight criteria, and another stricter, after excluding the possibility of flea hypersensitivity dermatitis, including ten criteria, are considered for the diagnosis of feline NFHD. By applying the first set of criteria, a sensitivity and specificity of roughly 75% is possibly obtained in the presence of five criteria. However, the second set would allow a sensitivity of 90% and a specificity of 83% for the diagnosis of NFHD, resulting in more reliable diagnosis of NFHD in cats.

Special attention should be given to specific patterns, mostly pruritus on the head and neck, miliary dermatitis, self-induced alopecia and eosinophilic lesions, but less frequent symptoms such as plasma cell pododermatitis, seborrhea, ceruminous otitis, facial erythema and exfoliative dermatitis have been reported.

In cats, specific patterns of skin reaction may indicate a primary allergic cause but are not specific for feline atopic syndrome (fAS). These patterns include the mentioned pruritus on the head and neck, miliary dermatitis, self-induced alopecia and eosinophilic lesions. Despite cutaneous manifestations being most prevalent in fAS, attention should be given to other extra-cutaneous clinical manifestations in allergic cats, like sneezing, coughing, sinusitis and conjunctivitis, diarrhea and vomiting as well as feline asthma (Gedon and Mueller 2018, Miller et al. 2013). However, the prevalence of these conditions in fAS lacks determination (Diesel 2020, Gedon and Mueller 2018, Favrot 2013).

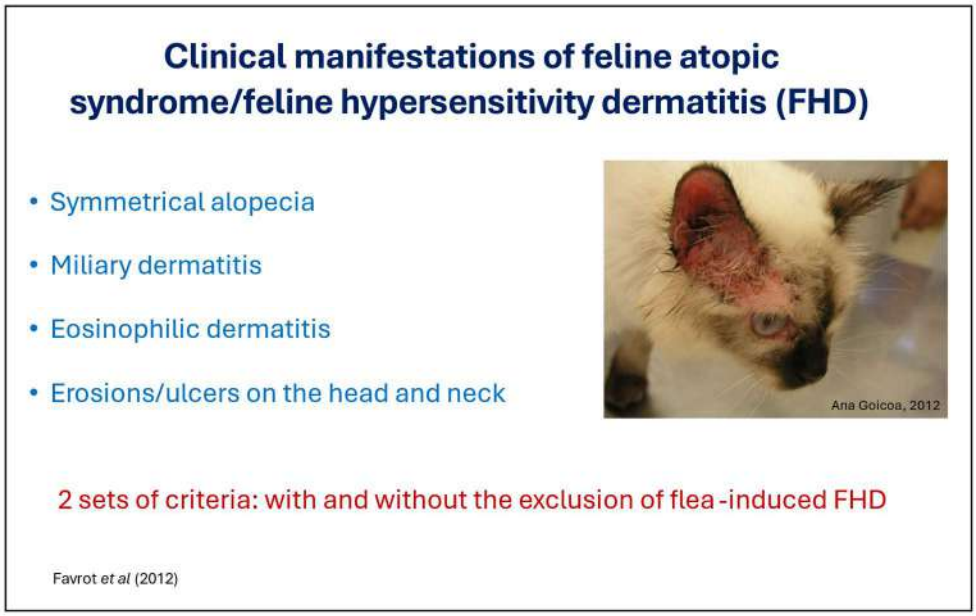
Horse consultation for allergy-related pruritic reasons has also increased during the past decades (White 2023). **Allergic dermatitis in horses** has been framed in, at least, one of the following conditions: 1. atopic dermatitis, 2. insect-bite dermatitis, 3. contact dermatitis, and 4. food allergy dermatitis, but other possible causes of pruritus should also be ruled out at the starting level of physical examination (White 2023, Marsella et al. 2023, Fadok 2013).

In the absence of a consensus regarding horse atopic dermatitis and suspecting that horse atopy is most likely to cause respiratory clinical signs like asthma, horse atopy is commonly assessed as an environmental allergic respiratory condition. Hence, after ruling out possible parasitic or infection causes of pruritic dermatitis, and primary respiratory infections, special attention should be given to possible atopy, mainly respiratory.

By the end of the class, a summary will be produced regarding cat and horse atopic conditions, and it is supposed that students will acquire the

essential knowledge, aiming at a diagnosis with high sensitivity and specificity.

Examples of slides to be used in Lecture 4:



Class 5

 # Feline and equine asthma:

- Diagnosis;

- Journal club 2: discussion of papers on the pathophysiology of asthma.

Cat and horse may develop a restrictive asthmatic bronchial condition close to the human equivalent, also in response to common sources as environmental allergens.

The first part of the class will be composed of a lecture addressing feline and equine asthma in terms of development and presentation, aiming to establish a positive and as far as possible complete diagnosis. The second part of the class will assume the format of a journal club regarding the matter of pathophysiology of asthma.

Feline asthma is a lower-airway inflammatory disease of cats, with allergic etiopathogenesis. It is related to aeroallergen-induced stimulation of Th2 response, followed by a complex pathway of cytokine liberation, leading to airway hyperresponsiveness with inflammation and airflow limitation (Trzil 2020).

Feline asthma is more frequently observed in young adults, affecting approximately 1-5% of the feline population. A chronic cough with possible exacerbations of dyspneic expiratory distress, in association with the characteristic abdominal "push", stands the most characteristic clinical frame.

To confirm the diagnosis of feline asthma, a thoracic imaging and the ruling out of other causes of respiratory distress and airway eosinophilic response, such as heartworm disease, is needed (Trzil 2020, Garrity et al. 2019, Nafe 2017). Affected patients may show diffuse bronchial or bronchointerstitial patterns, hyperinflation due to "air-trapping", and collapse of the right middle lung lobe, due to mucus obstruction. Nevertheless, up to 23% asthmatic cats will show normal thoracic imaging (Trzil 2020, Nafe 2017).

A differential diagnosis is often necessary regarding feline asthma and chronic bronchitis as both conditions share many clinical features such as chronic cough and a radiographical bronchial pattern. Chronic bronchitis is thought to derive from a previous airway insult, such as respiratory infections or inhaled irritants. For differential diagnosis, a cytology from bronchoalveolar lavage fluid (BALF) is frequently mandatory. While feline asthma is primarily characterized by eosinophilic inflammation (>17% eosinophils identified in BALF) an increase in the neutrophilic population is more frequently detected in BALF from cats affected by chronic bronchitis. Nevertheless, these differential findings are not always observed as chronic eosinophilic asthma may also evolve with airway damage with neutrophilic inflammation, resulting in a mixed inflammation (Trzil 2020).

A summary regarding a positive diagnosis of allergic asthma in cats will be emphasized – it should be based on: 1. detailed physical examination, 2. compatible thoracic imaging (radiography and computed tomography), 3. eosinophilic airway inflammation (through bronchoscopy and BALF), 4. ruling out possible heartworm disease (by a negative result for

antigens/antibodies), and 5. exclusion of other parasitic conditions such as aelurostrongylosis or toxocariasis (Trzil 2020, Nafe 2017).

Regarding the **syndrome of equine asthma** (SEA), a condition formerly known as recurrent airway obstruction (RAO) or inflammatory airway disease (IAD), it stands as a chronic, non-infectious respiratory condition of horses, characterized by inflammation of the lower airways in response to environmental allergens such as dust-mites, molds, or pollens.

Main clinical manifestations are: 1. chronic coughing (resting or exercising), 2. nasal discharge, 3. dyspnea (especially when expiring), 4. exercise intolerance or reduced performance, 5. tachypnea (with increased effort, and 6. observation of heave line (when severe).

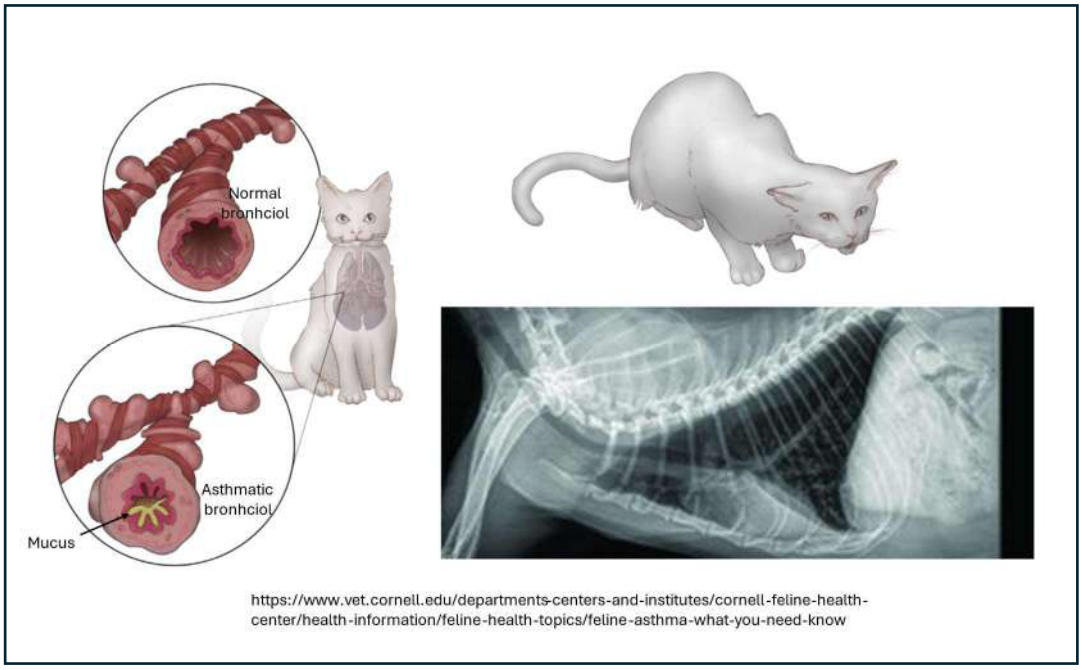
Diagnosis should be based on clinical manifestations and history, and bronchoscopy (with airway mucus observation), BALF cytology and pulmonary spirometry testing.

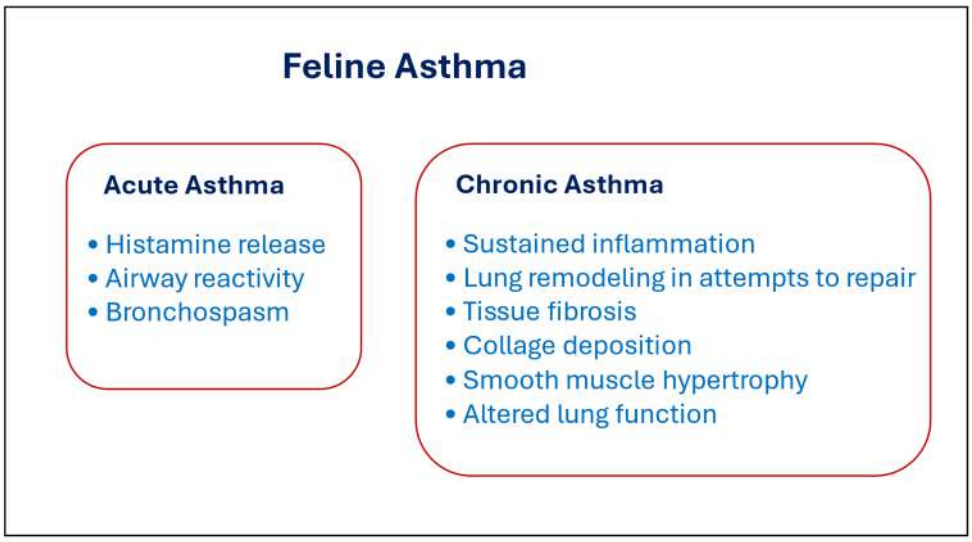
Upon a SEA diagnosis, further clinical investigation should proceed in order to classify the condition into one of two stages: 1. mild to moderate asthma, or 2. severe asthma. Mild to moderate asthma is more frequent in young to middle age, but can affect horses of all ages, causing occasional coughing and poor performance. Does not cause increased respiratory effort at rest, is associated with excessive mucus in the tracheobronchial tree, mild increase in BALF neutrophils, undetected or mild airflow limitation, and airway hyperresponsiveness. Regarding severe asthma, it usually affects horses older than seven years, with familial history of asthma, causes frequent coughing, exercise intolerance, history of exposure to dust or allergens, excessive mucus and moderate to severe increase in BALF neutrophils, airway hyperresponsiveness, and is reversible with bronchodilator or environmental change. Both conditions may show clinical signs variation over time (Couëtil et al. 2016).

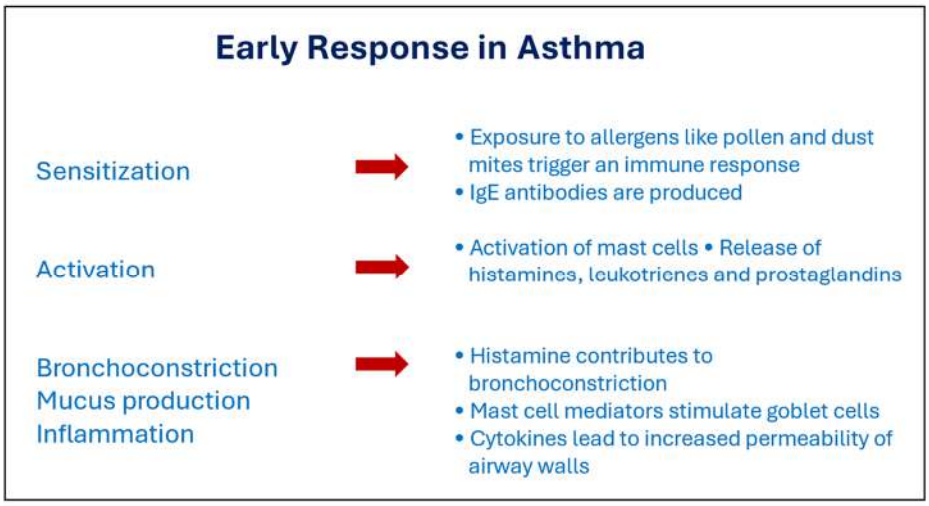
At the end of the lecture a summary of the main characteristics of cat and equine asthma will summarize this condition in both species.

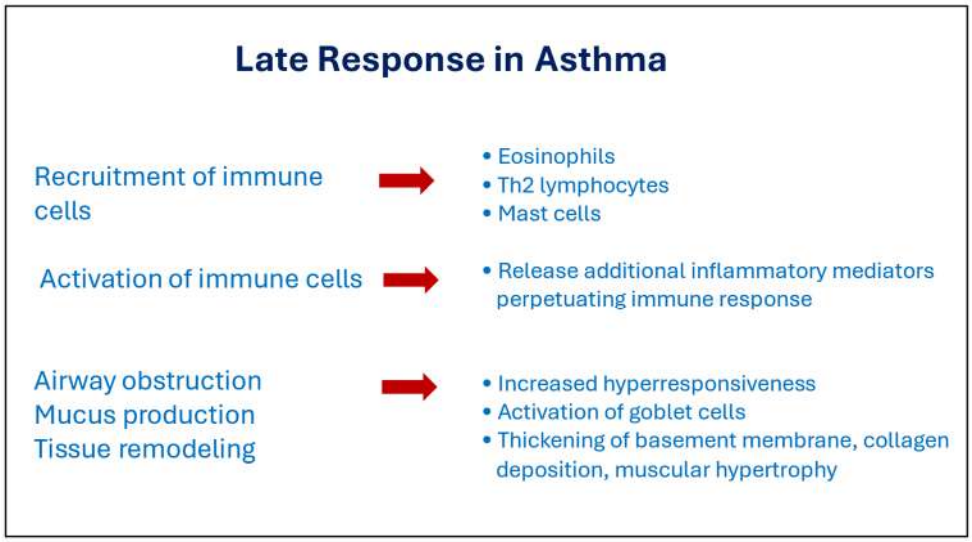
In the second part of the class, in a **journal club** format, 1-2 consensus papers addressing the pathophysiology of asthma (*e.g.* Trzill 2020, Nafe 2017, Couëtil et al. 2016) will be discussed, promoting student's further understanding of respiratory condition, aiming at a more rational diagnosis/ therapeutic approach.

Examples of slides to be used in Lecture 5:









Class 6

 # Ocular allergy

 # Food allergy

This class focuses on two main matters. The first is ocular allergy, which has been less diagnosed and addressed in clinical practice, despite the well-being compromise and the possible complications. The second is food allergy, which diagnosis is mainly obtained by exclusion, upon elimination diets. However, a common occurrence of environmental allergy (sometimes with ocular response) and food allergy may constitute a true diagnostic challenge, aiming a symptomatologic relief

as the patient will only recover when both conditions are well diagnosed, allowing an effective therapeutic approach. Hence, both conditions will be addressed for a better identification and characterization as an essential clinical objective.

Besides the previously referred type III hypersensitivity “blue-eye” condition, associated with canine viral hepatitis or the vaccination against, **allergic conjunctivitis**, mostly follicular, frequently associated with allergic rhinitis, have been related with the more common type I hypersensitivity (Delgado et al. 2023, Lourenço-Martins et al. 2011, Goicoa et al. 2008, Raffan et al. 2005, Arlian et al. 2003, Bensignor and Carlotti 2002, Lacey and Dutkiewicz, 1994). In fact, bacterial conjunctivitis has been one of the Willemse´s Minor Criteria for Canine Atopy, already in 1986 (Willemse, 1986), which could be associated with eye scratching or facial rubbing due to pruritus. Testing for allergy has already been studied in atopic dogs presenting conjunctivitis, by conjunctival provocation (Lourenço-Martins, 2011), and canine allergic conjunctivitis (cAC), which is frequently associated with cAD, was already shown related with the increase of interleukin (IL)-6 and IL-12, two Th2 cytokines detected from conjunctival biopsies (Varandas et al. 2020).

In cats, although cutaneous manifestations are the most prevalent in fAS, other extra-cutaneous clinical manifestations like sneezing, coughing, sinusitis as well as conjunctivitis, have been also reported (Gedon and Mueller 2018, Miller et al. 2013).

A systematic diagnostic approach is mandatory to identify allergic conjunctivitis and its possible complications, ruling out other primary causes as treatment approach could be rather different, avoiding misdiagnosis with possible severe consequences.

Gastrointestinal and cutaneous symptoms are frequently associated with **food allergy** (White 2023, Fadok 2013, Picco et al. 2008, Osborn 2006, Lloyd 2006), despite skin being the most frequent target-organ

involved in food allergy symptoms in animals. Hence, as well as ectoparasite infestations, infections, endocrine or autoimmune diseases, and allergy to flea bites, food allergy is also one of the conditions needing to be assessed when facing an atopic dermatitis suspicion (Olivry 2011, Olivry 2010, Olivry et al. 2010).

Food allergy dermatitis has been considered a condition somehow apart from atopic dermatitis (Martins et al. 2016), based on both etiological and clinical concepts, in accordance with the former definition of atopy, proposed by Halliwell in 2006: “A genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features. It is most commonly associated with immunoglobulin (Ig)E antibodies to environmental allergens”. However, quite recently, in 2024, definition of cAD has been updated by the ICADA, based on the most recent knowledge of its pathogenesis. Thus, cAD has been redefined as “A hereditary, typically pruritic and predominantly T-cell driven inflammatory skin disease involving interplay between skin barrier abnormalities, allergen sensitization and microbial dysbiosis” (Eisenschenk et al. 2024). The new definition is now covering the current broader understanding of the pathogenesis of cAD. In fact, “this new definition highlights the multiple factors involved in this disease, which supports the recommendation that the management of canine atopic dermatitis must be multimodal” (Eisenschenk et al. 2024) as it was already considered by Olivry et al. (2015). This new and more comprehensive definition may also cover those similar clinical patterns, observed in some cases where it is not possible to demonstrate the implication of IgE by allergological tests, leading to the diagnosis of atopic-like dermatitis (Martins et al. 2016, Halliwell 2006), which is frequently observed in food allergy, hence encompassing the condition in the cAD concept.

Food allergy dermatitis has also been classified as an allergic condition of horses (White 2023, Marsella et al. 2023, Fadok 2013).

When food allergy is suspected, by skin or gastrointestinal manifestations (Mueller and Olivry 2018), strict hypoallergenic diets should be implemented for roughly eight weeks as cutaneous and serological tests lack the necessary reliability in identifying the implicated food (Stapel and Kleine-Tebbe 2008, Hamilton and Adkinson 2004). If clinical manifestations markedly decrease, individual food provocation – Oral Food Challenges (OFC) – should be performed to identify the implicated food (Olivry and Mueller 2020, Mueller et al. 2016). However, prior to an elimination diet, a previous patch testing, like it is performed to diagnose specific contact dermatitis (Ho et al. 2015), may help identifying the possible implicated food species, which could be useful to include since it has been reported to show reasonable negative predictability (Mueller and Unterer 2018).

Recent studies, performed by Olivry et al. (still unpublished) suggest the usefulness of lymphocyte proliferation tests, following OFC, for the diagnosis of delayed food allergy in dogs. It may help differentiating delayed from immediate food allergy, a suspected IgE-mediated condition. A delayed food allergy response (suspected of a cell-mediated mechanism) seemed to be the most common type of food allergy in dogs, since only 9% of dogs submitted to OFC with the implicated food, showed a return of symptoms during the first day upon challenge (Olivry and Mueller 2020).

At the end of the class, a summary highlighting diagnostic pathways and the relevance of identifying ocular allergy and food allergy will be made. Students should be able to identify conjunctivitis, assessing their possible allergic origin, and equate the diagnostic pathway regarding the identification of food allergy, either as an isolated or combined clinical condition.

Examples of slides to be used in Lecture 6:

Class 7

 # Allergy to insect bites in horses

 # Contact allergy

This class focuses on allergy to insect bites, a common condition presented by some horse patients, with culicoides midges as the most frequent allergenic species. Identification of this condition is rather important because of health implications, which are truly relevant for affected horses. Important research has been developed, lately.

Contact allergy, the other focus of this class, is a condition accepted to be triggered by a type IV hypersensitivity. This inflammatory, pruritic skin reaction, occur upon prolonged contact with allergenic substances, some of them probably haptens. Sometimes, the causative material is not easily identified, requiring special attention and time to identify the causative material. Sometimes, complementary diagnostic methods such as patch tests may be useful.

Insect Bite Hypersensitivity (IBH) is known as a recurrent seasonal pruritic dermatitis, affecting a worldwide variety of horses, and has been framed as one of the implicated causes of allergic dermatitis (White 2023, Marsella et al. 2023, Fadok 2013). Resulting mainly from type I hypersensitivity, it may also occur due to type IV hypersensitivity (Marsella et al. 2023, Jonsdottir et al, 2019, Anderson, 1993). However, the condition is accepted as a multifactorial disease, affecting different breeds, with a prevalence ranging from 5 to 10% (Schaffartzik et al. 2012, Schurink et al. 2011).

There are citations related to insect-bite hypersensitivity for over 160 years. Some authors referred to as an intense pruritic condition with lesions distributed along the mane, withers, back and tailhead, associated to allergic reaction to insect bites, following insect bite hypersensitivity (IBH), and others as summer sores, in association with larvae from *Habronema* spp. (Marsella et al. 2023).

The triggering allergens are inoculated into the horse's skin, through the saliva of female *Culicoides* spp., which are hematophagous, when taking their blood meal. The insects may mechanically damage the skin, and components of the saliva, like different enzymes such as hyaluronidase, trypsin, and chymotrypsin (Russel et al. 2009, Wilson et al. 2008) will also contribute to the skin barrier disruption and to the digestion of the blood (Marsella et al. 2023).

During the rise and dusk periods, when *Culicoides* show higher activity, horses may be bitten by a large number of female insects, and during long

periods, contributing to sensitization, first, and allergy-triggering, afterwords, consisting in a truly enhancing process (Marsella et al. 2023).

The main *Culicoides* spp associated with IBH are *C. nubeculosus* and *C. obsoletus*. Different species show different environmental and food preferences, contributing to different incidence rates around the world (van der Rijt et al. 2008, Anderson et al. 1993).

Manifestations in allergic horses usually start between two to four years of age and occur seasonally, according to insect activity, which happens from Spring to Autumn in temperate climates, mainly during rise and dusk (Scott and Miller 2003).

Allergic horses will present pruritus, self-induced cutaneous lesions as papules, usually evolving to crusts, which may infect, aggravating pruritus and consequently the lesions (Marsella 2019). Despite the affected areas may vary according to the insect species (van der Rijt et al. 2008), lesions commonly present a dorsal distribution, from the mane to the base of the tail, but other areas like face, neck, shoulders and low thorax and abdomen may also be affected (Fadok 2013, Scott and Miller 2003).

Chronic pruritic affection, frequently associated with recurrency, may lead to extensive alopecic crusty lesions with lichenification and leukotrichia (Pessoa 2023, Marsella et al. 2023).

Besides skin tests and specific IgE determination in serum, laboratory methods such as Cellular Antigen Stimulation Test (CAST), used to evaluate the release of inflammatory mediators like sulfidoleukotriene (sLT) from allergen-stimulated peripheral blood leukocytes (Baselgia et al. 2006) has shown to be useful when investigating allergy to *Culicoides* in horses (van der Meide et al. 2012, Langner et al. 2008) as sLT are more frequently released in IBH horses than in healthy controls (Mueller et al. 2016). In this context, a high correlation between *in vitro* sLT and histamine release from peripheral blood leukocytes was observed

following stimulation with *Culicoides* allergens (Marti et al. 1999) suggesting that the sLT release could be used for *in vitro* detection of immediate-type I reaction.

Contact allergy or **contact allergy dermatitis** is also a possible allergic condition, either in small animals (Martins et al. 2016) or in horses (White 2023, Marsella et al. 2023, Fadok 2013). It is characterized by an inflammatory, pruritic skin reaction, occurring upon prolonged contact with allergenic substances.

The diagnosis of contact allergy dermatitis is frequently made by exclusion of other chronic or recurrent pruritic causes, including atopy. However, patch testing has been indicated as a method for investigating possible delayed hypersensitivity, like contact allergy dermatitis. A patch testing approach has been advised prior to starting an elimination diet, like it is recommended for specific contact allergy dermatitis diagnosis (Ho et al. 2015), as it may help identify the possible implicated species. In fact, it has been reported to show reasonable negative predictability (Mueller and Unterer 2018).

When diagnosing suspected allergy contact dermatitis, after discarding other possible inflammatory and pruritic conditions, like associated to infectious and parasitic causes, irritant contact dermatitis, caused by cleaning products, different materials or even medicinal preparations, should be also equated.

Different kinds of materials and substances are currently known as causing delayed hypersensitivity reactions, like plants, topical medicines and flea-collars, plastic objects, and different fabrics.

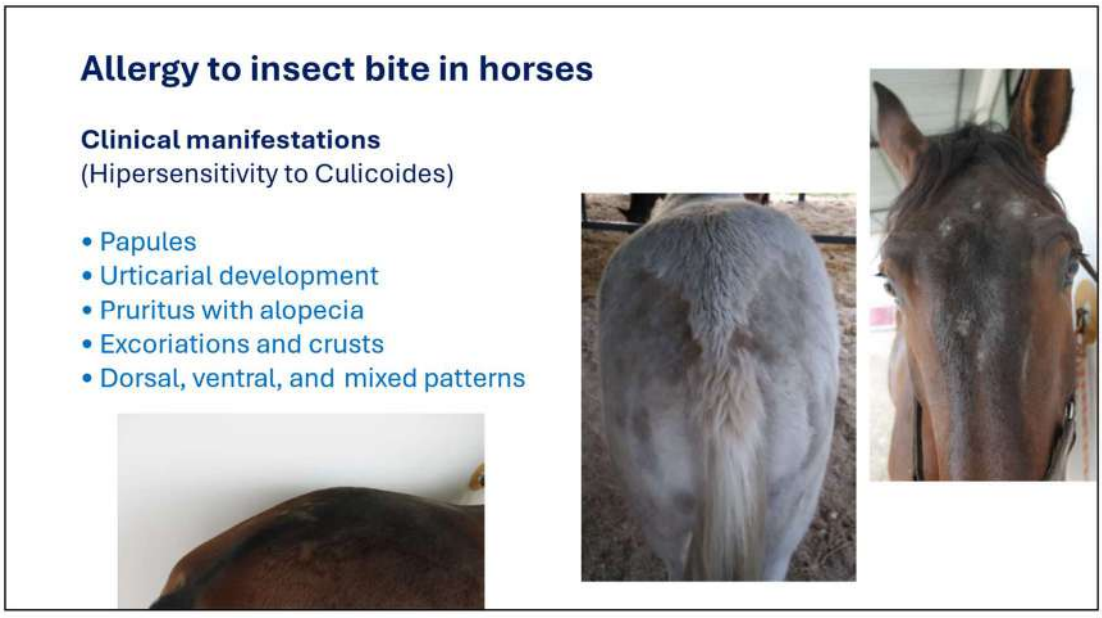
The condition is relatively uncommon because of the protective barrier provided by the fur coat. That is why most symptoms develop in glabrous zones or with sparse hair, like muzzle, around the eyes, armpits, elbows ankles, belly, scrotum and paws. The more common symptoms include

inflammatory skin, alopecia, scaling, darkening, pustules, and scratching (Kearley 2025).

In summary, when facing a suspicion of allergic contact dermatitis, associated with the distribution of skin lesions and contact surfaces, diagnosis should start by discarding other possible causes and search for what may get in contact with those affected areas. Then, further diagnostic investigation may be performed by patch testing.

At the end of the class, a global summary highlighting the relevant issues in the diagnostic approach of IBH and contact allergy will be made. Students should be able to identify IBH and produce confirmative diagnostic, and equate a diagnostic protocol for contact allergy.

Examples of slides to be used in Lecture 7:



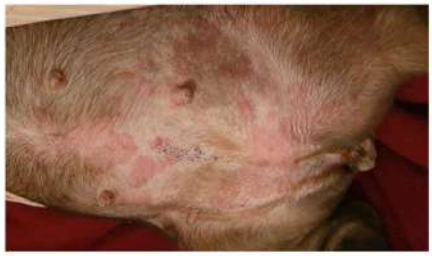
Allergy to insect bite in horses

Diagnostic specificities associated with the agent

Group	Breeding environment	Feeding time
Horse flies, deer flies	Vegetation and water	Daytime feeders
Horn flies	Cattle	Daytime feeders
Stable flies	Rotting vegetation	Daytime feeders
Black flies	Running water	Morning and evening
Culicoides	Standing water, manure/vegetation	Twilight to dawn
Mosquitos	Water	Dusk to 2h past sunset

White, 2015

Contact Dermatitis



- Frequent observations → Inflammatory, pruritic skin reaction, upon prolonged contact with allergenic substances
- Diagnosis → Frequently by exclusion of other chronic or recurrent pruritic causes, including atopy; Patch testing
- Pathophysiology and materials → Delayed hypersensitivity reactions to plants, topical medicines, flea-collars, plastic, and different fabrics

Class 8

 # Identifying and characterizing allergens

This class focuses on allergen identification as a basis for the determination of individual allergograms, the basis of a CRD, which in turn stands essential for the precision medicine in immunoallergology (Matricardi et al. 2025; Passalacqua and Canonica 2015).

As imunoallergology evolves in both human and animal fields, more allergens are being tested as they are found relevant for sensitization and allergy. Some of them are still to be identified as allergens, with possible

cross-reactions between them, making the identification and characterization of those molecules, of crucial importance for the ongoing diagnostic evolution. A panorama of the allergomes for different animal species will be addressed.

An allergen is basically an antigen that can cause an allergic reaction. In some individuals, the immune system recognizes allergens as foreign or dangerous, reacting by producing IgE to defend the organism, and this reaction leads to allergy (Medical Encyclopedia 2025, Hamilton 2019). In this context, the discovery of IgE, in 1967, made possible the identification of allergens as the substances implicated in the pathophysiology of allergic conditions. This allowed the evolution of the diagnostic process, becoming more precise and mirroring the advances in both allergen identification and pathophysiology.

Considering that different patients may show sensitized and allergic to different allergen species, a coherent diagnosis of allergy – yes or no – should be previously made as not much difference is usually found regarding allergen-specific IgE frames, between atopic and nonatopic dogs (Layne and DeBoer 2019). Then, facing an allergic patient, when ASIT is considered a suitable option, identifying the species to which sensitization occurs is essential for the currently called specific immunotherapy. However, besides the species to which a patient is sensitized to, identifying the molecular allergens from among those species allergomes, that are specifically sensitizing to that patient, becomes truly important when aiming at a rather effective ASIT (Olivry et al. 2024). Furthermore, the current knowledge regarding the clinical relevance of IgE may also play a relevant role in allergy presentation (Kumagai et al. 2021).

In the context of the current knowledge, it is considered highly relevant to identify the molecules from among the species' allergomes, to which an individual is sensitized in a relevant way, assuring their inclusion in an effectively specific immunotherapy. In fact, from among different

sensitizing species, it is estimated that roughly 2000 allergens may be relevant regarding dogs and 700 to cats. Furthermore, from the proteome of around 11000 molecules of the *Dermatophagoides farinae* dust mite, only a figure of just 40 may reveal relevant regarding dog sensitization (Nextmune – unpublished data).

The updated information related with the identified molecular allergens is available at two main databases: 1. "allergome.org", and 2. "allergen.org", this one by the International Union of Immunological Societies (IUIS) – Allergen Nomenclature Sub-Committee, approved by the World Health Organization. It includes the systematic nomenclature of allergenic proteins with their approved allergen names. Successive updates of allergen names, approved at the meetings of the Sub-Committee, reflect the progress of identification, cloning, and sequencing of allergens (Radauer et al. 2014), which is an ongoing process.

Research on veterinary allergy diagnosis is currently speeding up with the launch of the PAX®, a state-of-the-art methodology from Nextmune (Stockholm, Sweden), using the Macroarray Diagnostics (MADx) technology (Macroarray Diagnostics, Vienna, Austria). This multiplex diagnostic tool is now available for dog, cat and horse complementary laboratory allergology approach. With the 2023 version of PAX®, dog sera may be tested against 247 allergen spots, composed of 75 individual extracts, two mixes of two extracts, 169 individual components, and one two-component mix. Environmental aeroallergens occupy 132 of the spots, with 39 individual extracts, two two-extract mixes, and 91 components. Hymenoptera venoms occupy 13 spots, with five individual extracts and eight individual components. Ninety-eight spots are occupied by food allergens, with 31 individual extracts, 66 individual components, and one two-component mix (Olivry et al. 2024a). A second and updated version of the PAX® is expected in the second half of 2026.

Allergen research for diagnosing sensitization and allergy in animals has started with allergens already known to humans. However, as many molecules may be found allergenic to animals, further investigation to identify other relevant sensitizing molecules to animals is needed. This basal research of allergen identification and characterization should proceed through protein extraction, separation by isoelectric point and molecular weight, followed by testing against animal serum for IgE recognition (Martins et al. 2017, Martins et al. 2015).

By identifying and characterizing allergens at the molecular level, a new era in veterinary allergy diagnosis will be opened, allowing extensive identification of primary *vs* cross-reactive sensitization, through CRD, contributing to further effectiveness of immunotherapy by narrowing the range of allergy-triggering allergens to be administered in a more specific immunotherapy. In fact, this immunotherapy with the more adequate pool of allergens for each individual as a tailor-made CRIT, aims at a more specific and therefore effective approach (Matricardi et al. 2025; Canonica et al. 2013, Valenta et al. 1999).

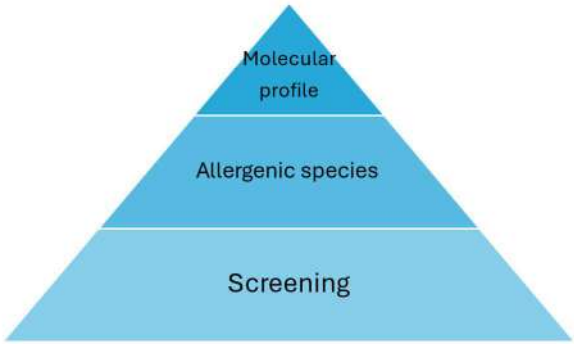
Illustration of different methods for allergen identification and characterization, by protein extraction and standardization, and separation by isoelectric focusing (IEF) and one or two-dimensional sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE), followed by serum IgE characterization (Western-Blotting) will be explained and results exemplified.

At the end of the class, a summary highlighting the diagnostic and therapeutic purposes of allergen identification and characterization will be made beyond the species level.

Examples of slides to be used in Lecture 8:

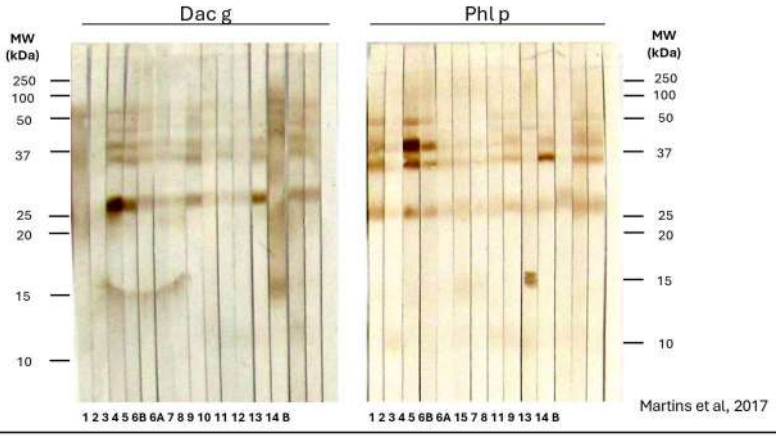
Allergen identification – Levels of characterization by IgE

CRD (**C**omponent **R**esolved **D**iagnosis)

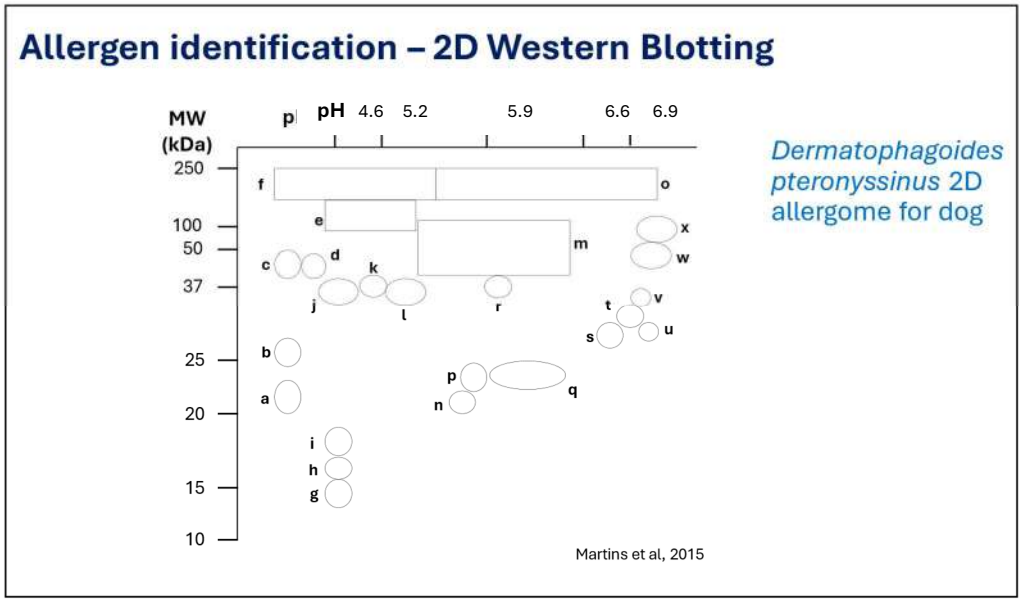


Allergen identification – Western Blotting

Allergens from *Dactylis glomerata* (Dac g) *and Phleum pratense* (Phl p) for dog



Martins et al, 2017



Class 9

#Complementary methods in allergy diagnosis – Clinical and laboratorial methods

This class focuses on different clinical and laboratory diagnostic methods for allergy diagnosis, promoting the understanding of their advantages and limitations, for a better integrated selection.

After a clinical diagnosis of allergy, either to environmental or food allergens, complementary methods should be used to help identify the implicated species. Complementary clinical methods are currently based on **cutaneous (intradermal or prick) tests** and have shown valuable for the identification of sensitization to species such as dust mites, pollens and molds (Pali-Schöll et al. 2020, Mueller et al. 2018, Martins et al. 2016, Halliwell 2006). Regarding food allergy, despite the possible usefulness of intradermal and serological tests, when sIgE is found implicated (Martins et al. 2019), **exclusion/hypoallergenic diet** is the golden standard diagnostic method for both skin and gastrointestinal manifestations (Mueller and Olivry 2018). These diets should be implemented for eight weeks and be followed by **Oral Food Challenge (OFC)** with the most likely implicated foods, like beef, dairy, chicken,

wheat and lamb, regarding dogs, and beef, fish and chicken, regarding cats (Olivry and Mueller 2020, Mueller et al. 2016). An OFC should last up to two weeks, to allow a proper diagnosis of delayed food allergy. Relapsing of clinical signs are expected after one day in 9% of dogs and 27% of cats, up to two days in 21% of dogs and 29% of cats, between five to 14 days in 50% of dogs, and between four to seven days in 90% of cats (Mueller et al. 2016).

As for contact dermatitis diagnosis (Ho et al. 2015), **patch testing** may also be useful to discard allergy to specific implicated food species, since it has been reported to show reasonable negative predictability (Mueller and Unterer 2018).

Despite its experimental characteristics, **histamine release following basophil degranulation** has been observed in response to blood cell stimulation with *Culicoides* extracts, in both insect-bite hypersensitive (IBH) and healthy horses (Wagner et al. 2008, Baselgia et al. 2006). However, histamine release test may be object of further standardization, which may help diagnose severe food allergy (Prélaud et al. 1998) or reactions to common drugs (Pineda et al. 2015).

While the **basophil activation test (BAT)** may help diagnosing cases of IgE-mediated food allergy (Ocmant et al. 2009), standing as a safe alternative to OFC (Nopp et al. 2009), determination of **eosinophilic cationic protein (ECP)** may be useful to monitor the allergic state also in animals.

Another method commonly used to investigate drug allergy in humans, that has also been applied to different animal species (Pichler and Tilch 2004, Barta and Oyekan 1981), is the **lymphoblastic transformation test (LTT)**. However, for a useful application to veterinary purposes, it should undergo further standardization in terms of physiological response ranges (Palma et al. 2009).

The **Cellular Antigen Stimulation Test (CAST)** is a method used to evaluate the release of inflammatory mediators like sulfidoleukotriene (sLT) from allergen-stimulated peripheral blood leukocytes (Baselgia et al. 2006). The CAST was used to investigate allergy to *Culicoides* in horses (van der Meide et al. 2012, Langner et al. 2008) as sLT are more frequently released in IBH horses than in healthy individuals (Mueller et al. 2016). As a high correlation between *in vitro* sLT and histamine release from peripheral blood leukocytes had been observed following stimulation with *Culicoides* allergens, sLT release could be used for *in vitro* identification of type I hypersensitivity reaction (Marti et al. 1999).

Until recently, regarding laboratory determination of allergen-specific IgE in animals, **enzyme-linked immunosorbent assay (ELISA)** (Lee et al. 2009) has been the only reliable commercially available method. In this method, comprising different variants, allergens are coated into the wells of an ELISA-plate, and sera dilutions are subsequently incubated with the allergens. Then, sIgE fixated to the allergens is revealed by a secondary antibody (or an antibody cocktail) enzymatic conjugate, promoting a color-developing result, proportional to the serum concentration of sIgE.

A real step forward is currently available for serologic diagnosis of sensitization in veterinary medicine. Using the Macroarray Diagnostics (MADx) technology (Macroarray Diagnostics, Vienna, Austria) Nextmune (Stockholm, Sweden) launched the **Pet allergy Xplorer (PAX®)**, a multiplex diagnostic tool, available for dog, cat and horse complementary laboratory allergology approach, with dozens of allergens from different species, between native and recombinant.

The 2023 version of the PAX® presented 247 allergen spots, composed of 75 individual extracts, two mixes of two extracts, 169 individual components, and one two-component mix. Environmental aeroallergens occupied 132 of the spots, with 39 individual extracts, two two-extract mixes, and 91 components. Thirteen spots were reserved for

Hymenoptera venoms, with five individual extracts and eight individual components. Food allergens occupied 98 spots, with 31 individual extracts, 66 individual components, and one two-component mix (Olivry et al. 2024a). The PAX® method prevents determining high sIgE scores against components lacking clinical relevance, like cross-reactive carbohydrate determinants (CCD), by previously dilution of sera with a buffer containing a mix of proteins with CCD motifs, besides including CCD detectors and their respective non-CCD controls. After CCD-IgE block, if an IgE level against CCD-bearing detectors is detected above a defined threshold, an alert arises for the possible lack of clinical relevance of the sIgE figures against either plant pollens, plant food extracts or native plant components (Olivry et al. 2024a). Hence, PAX® allows not only the identification of each patient's sensitization profile, but possible cross-reactions, differentiating primary from secondary sensitization, which may be relevant when determining what to include in the ASIT pool. Soon, this CRD may also allow veterinary allergists to prescribe a rational CRIT, aiming for higher therapeutic success.

Another method with possible usefulness for diagnostic investigation of specific cases of allergy is **immunophenotyping**. At the Th1/Th2 paradigm level, by measuring intracellular cytokines using flow cytometry, after stimulation with molecular allergens, Martins et al. (2008) showed that immunophenotyping could help interpretate specific cases of allergy.

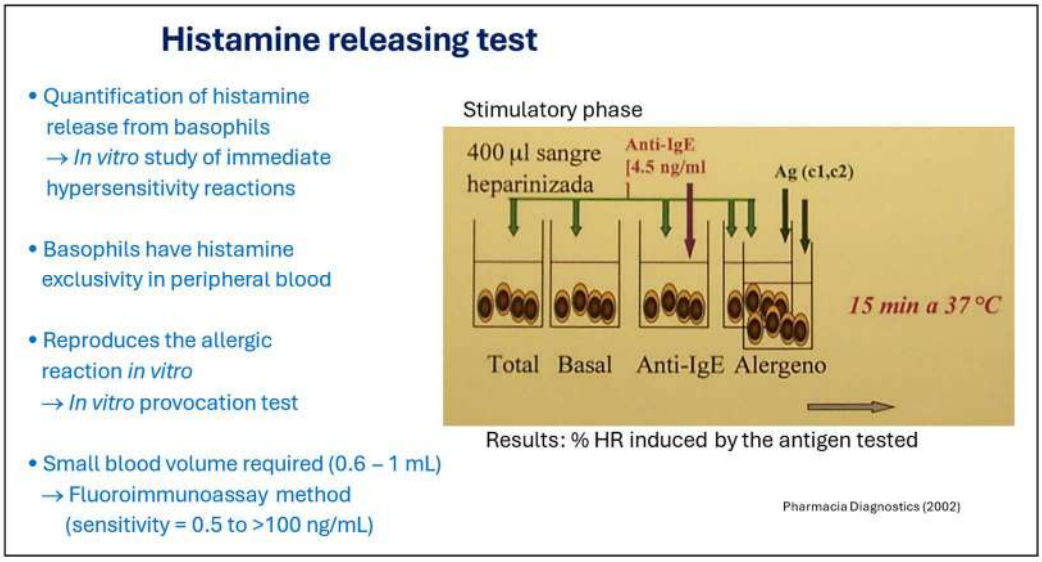
In 2011, Fugimura et al. conducted studies of lymphocyte proliferative response to food allergens in food allergic dogs, using flow cytometry, suggesting the validity of the method to differentiate between allergic and healthy animals. However, differentiation between cases of food allergy and atopic dermatitis is still a challenge, needing further development in veterinary medicine.

Immunophenotyping-based studies, especially regarding T regulatory cells (Tregs) following immunotherapy, are also increasingly promising

and may represent a helpful *in vitro* research approach in the allergy field as a marker of favorable/unfavorable immune response to allergen immunotherapy (Palomares et al. 2013, Roland et al. 2010).

At the end of the class, a summary will be presented with the different complementary clinical and laboratory diagnostic methods, highlighting their historic roles and main advantages for precision diagnosis. Students will be able to choose the more useful combination of methods for each patient and owner.

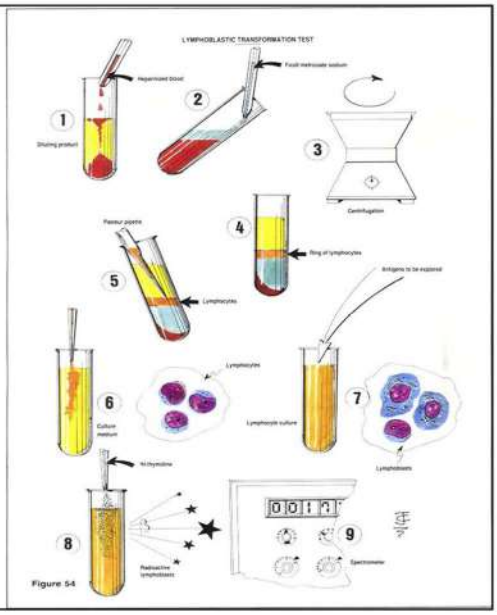
Examples of slides to be used in Lecture 9:



Lymphoblastic Transformation Test (LTT)

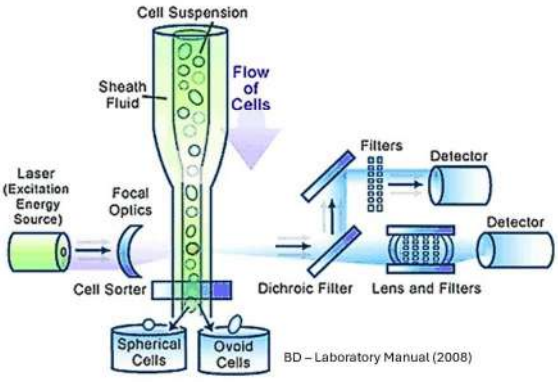
- Already sensitized lymphocytes undergo blast regression and proliferate when re-exposed to that antigenic stimulus.
- Culture of lymphocytes in the presence of suspected antigens.
- Assessment of proliferative blastogenesis by incorporation of ^{3}H-thymidine.
- Broad field of application.

Pharmacia Diagnostics (2002)

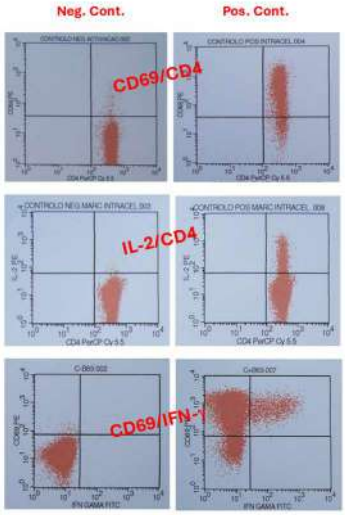


Immunophenotyping

Detection by Flow Cytometry



BD – Laboratory Manual (2008)



Martins et al, 2009

Class 10

 # Complementary methods in allergy diagnosis: Intradermal testing

This class focuses on intradermal testing, a fundamental method to identify sensitization/allergy to a given species. Despite its simplicity, this method, mimicking the natural contact of allergens with the immune system, demands a rather important set of rules for the obtention of reliable results, which will be assessed.

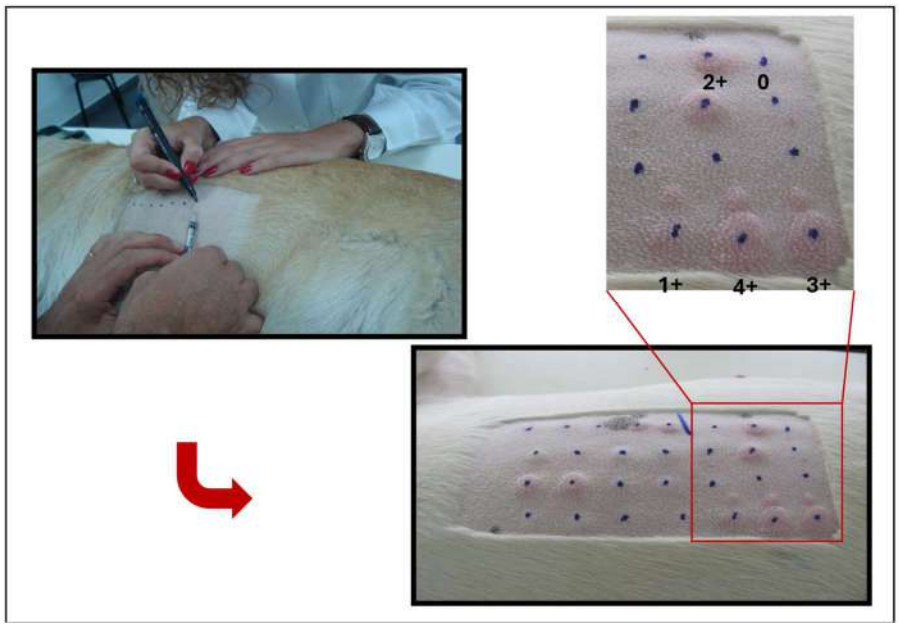
Intradermal testing (IDT) is a useful way to mimic the allergy-triggering reaction upon the contact of the allergens with patients' mastocytes. In a patient sensitized to the tested species, inoculated allergens will connect to the specific IgE fixed to their high affinity receptors (FCεRI) on the mastocyte surface, leading to the cross-link cell activation with degranulation and histamine release. Then, a local weal and flare congestive-edematous response will occur and be read.

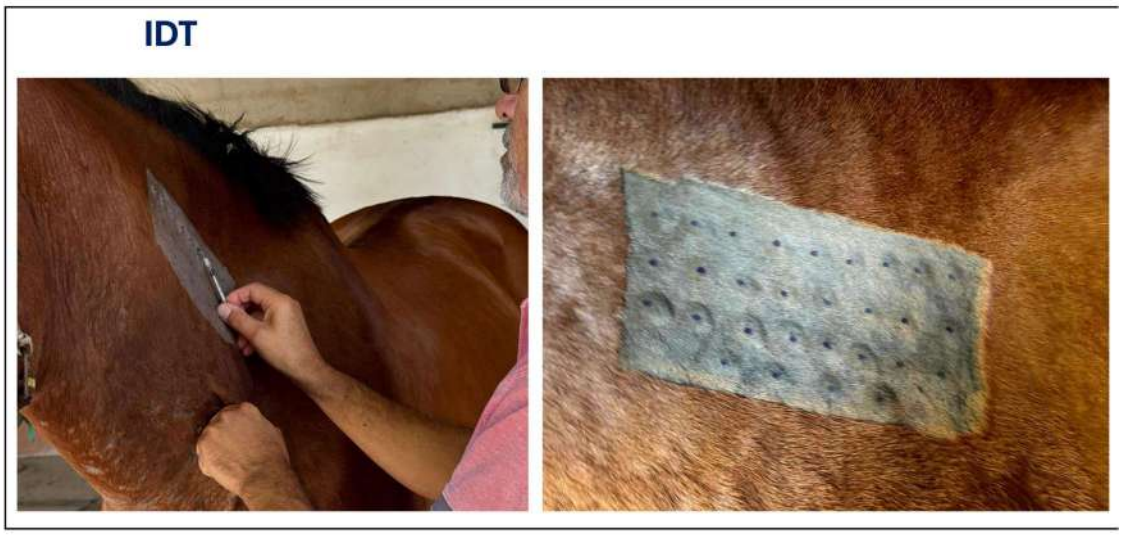
For IDT in dogs and cats, 50 µL (0.05 mL) of different commercial allergenic extracts and controls (positive: histamine solution; negative: solvent for extracts) are inoculated intradermally. The reaction (edematous papule) is read after 15-30 minutes. In cats, positive response may occur faster (e.g. in 5 min.), being advised from the moment of inoculation. In horses, the volume to be inoculated is usually increased to 150-200 µL (0.150-0.200 mL).

A positive reaction is considered when the observed wheal is at least equal or greater than halfway between the negative and the positive control reaction. When the subjective evaluation is used, which happens in the common practice, the positive control will assume a conventional grade of 4, whereas the negative control will be graded as 0. An IDT response is considered positive if it is graded as 2 or greater. A grade 1 response should be considered doubtful. Furthermore, withdrawal of several medicines should be observed for a minimum specific period, before testing (Hensel et al. 2015).

In this class, students will observe and/or practice IDT in patients from immunoallergology outpatient consultation, observing the results and equating the diagnosis.

Examples of slides to be used in Lecture 10:





Class 11

 # Therapeutic approach to allergy in dogs, cats and horses

 # Journal club 3: addressing the skin barrier

This class will address the subject of allergy therapy, which should aim at the cause, the symptoms and the feasibility of treatment, according to different factors. The tendency to chronicity will stand as a relevant factor when equating prolonged prescriptions, multimodal approaches being advised to avoid side effects, as much as possible.

Allergy therapy aims the healing, relieve or avoid clinical complications of an allergic condition. It is necessary to bear in mind the kind of allergy the patient presents, to manage the symptoms and avoid the allergen sources.

Regarding the kind of allergy, environmental, food, insect or contact allergy should be identified in association to manifestations like pruritus, otitis, skin erythema, sneezing or coughing, or even digestive signs such as vomiting and diarrhea.

In fact, as there is not "the diagnostic method" (Martins et al. 2016), there is not a single therapeutic protocol as well (Olivry et al. 2015).

Several main treatment approaches are currently identified as for: i) insect avoidance and population control; ii) inflammation control (antihistamines, glucocorticoids and omega-3 fatty acids); iii) immunomodulation (cyclosporin, oclacitinib, atinvicitinib, ilunocitinib and lokivetmab); iv) dietary management (elimination diets); v) environmental management (for dust-mite and pollen avoidance); vi) topical treatments (for skin hygiene and barrier improvement), and vii) allergen-specific immunotherapy (for remodeling the immune system response).

In general, and according to Olivry and Banovic (2019) the therapeutic approach may be divided into two main phases: **Reactive Therapy** (to

induce remission) and **Proactive Therapy** (to prevent recurrences). The first phase (reactive therapy) is mainly directed to acute situations, in which a stopping approach is recommended regarding the severity of the conditions, which demand urgent measures to restore well-being, and to avoid further complications and extensive development of immunological memory. In this phase, therapy frequently focuses on large breadth drugs as glucocorticoids, progressively narrowing the focus to immune modulating drugs as oclacitinib.

The second phase consists in a shorter breadth proactive therapy approach, following previous improvement, and acts to prevent recurrency. It should rely on allergen avoidance and on allergen-specific immunotherapy, when possible. Lokivetmab as well as topical glucocorticoids and oclacitinib, when needed, should be administered to keep allergy under control with minimum interference on ASIT-derived immune remodeling. If necessary, despite compromising the intended objectives of ASIT, cyclosporin and systemic glucocorticoids may and should be used in case of a possible severe relapse.

Therapeutic approaches in allergy may also be summarized in short- and medium-term, and medium- and long-term therapies, as follows, emphasizing the common relevant issues associated.

1. **Short- and medium-term therapies:**

    a) Identification and elimination of allergenic causes – Presence of ectoparasites, environmental conditions, secondary infections, and food (Marsella and Benedetto 2017, Olivry et al. 2010).

    b) Restoration of the skin barrier – Baths with non-irritating antiseptic, antifungal and antipruritic products, and containing proceramides (Paixão et al. 2022, Marsella 2013).

    c) Topical glucocorticoids – Reduce itching and localized skin lesions. Skin atrophy is the main side effect (Olivry et al. 2015, Olivry et al. 2010).

d) Systemic glucocorticoids – As hyperadrenocorticism may develop, if used for prolonged periods, short-term oral therapy may be preferable (DeBoer 2014).

e) Oclacitinib (Apoquel®, Zoetis) – Janus kinase 1 inhibitor; inhibition of IL-31-induced signal transduction; reduced side effects (Marsella et al. 2025, Gonzalez et al. 2024) as well as Ilunocitinib (Zenrelia®, Elanco) (Forster et al. 2025) or Atinvicitinib (Numelvi®, MSD) (Rahway 2025).

f) Antihistamines – Should be administered before the crisis (Gedon and Mueller 2018, Olivry et al. 2010).

g) Lokivetmab (Cytopoint®, Zoetis) – Caninized McAb, anti-IL-31, key interleukin in triggering pruritus (compatible with ASIT) (Moyaert et al. 2017, Michels et al. 2016).

2. Medium- and long-term therapies

a) Identification and elimination of allergenic causes (Hightower et al. 2010).

b) Avoiding contact of the animal with environmental allergens (Strzelczyk et al. 2020, Tovey 2008).

c) Restoration of the skin barrier – Baths (Olivry et al. 2015) and essential fatty acids (Marsella 2025, Paixão et al. 2022, Olivry et al. 2015, Olivry et al. 2010).

d) Antihistamines – Limited action (Saridomichelakis and Olivry 2016, Eichenseer et al. 2013).

e) Cyclosporine (Archer et al. 2014, DeBoer 2014).

f) Oclacitinib (Barreiro et al. 2025, Marsella et al. 2023a, Olivry et al. 2015), Ilunocitinib (Forster et al. 2025), or Atinvicitinib (Rahway 2025).

g) Lokivetmab (Barreiro et al. 2025, Kasper et al. 2024, Souza et al. 2018).

h) Topical glucocorticoids (Saridomichelakis and Olivry 2016; Olivry et al. 2015, Olivry et al. 2010).

i) Tacrolimus – Safer alternative to topical glucocorticoids (Olivry et al. 2015, Olivry et al. 2010).

j) Systemic glucocorticoids – Not recommended for long-term use (Saridomichelakis and Olivry 2016).

k) Pentofixillin associated with fatty acids (Olivry et al. 2015).

l) Allergen-specific immunotherapy (Pali- Schöll et al. 2020, Mueller et al. 2018, DeBoer 2017, Loewenstein and Mueller 2009).

Specifically, regarding **contact dermatitis**, main therapeutic approach should rely on avoiding contact with allergenic sources (Kunkle 1988), oral or topical administration of glucocorticoids (Marsella 2013) and systemic pentoxifylline (Marsella 2013, Marsella 2012) and topical tacrolimus (Saridomichelakis and Olivry 2016, Marsella, 2012).

New therapeutic approaches are also under the research focus, either in human or veterinary medicine as follows:

a) Probiotics (Gedon and Mueller 2018).

b) Vitamin D (Alizadeh et al. 2022).

c) Anti-IgE monoclonal antibodies (Hammerberg and Eguiluz-Hernandez 2017) (*e.g.* in human medicine: Omalizumab – Xolair®, Novartis Pharma – Djukanović et al. 2024).

d) Intralymphatic immunotherapy (Mueller et al. 2018); more specific and individualized (Martins et al. 2016).

e) Combined approaches (Gedon and Mueller 2018).

f) Anti-IL-31/IL-31 receptor vaccine – Robust specific IgG response, allowing significant reduction of pruritus in dogs allergic to dust-mites, subjected to provocation test (Li et al. 2023, Fettelschoss et al. 2020, Bachmann et al. 2018).

g) Canine anti-IgE vaccine (Bachmann et al. 2018).

h) More specific and effective immunotherapy (Moya et al. 2021, Plant and Neradilek 2017).

Specifically, regarding **cat respiratory conditions**, different complementary approaches may be used. As well as for skin acute conditions, corticosteroids will be key medicines when reducing airway inflammation is urgently needed, besides oxygen therapy. Prednisolone is the first-choice corticosteroid for systemic administration, while fluticasone would be the choice for inhaled maintenance administration. Bronchodilators like albuterol and salbutamol are also useful inhaled resources, which may be administered using spacer chamber and mask (AeroKat®, Trudell Animal Health, London, ON, Canada) or like terbutaline, systemic. Inhaled administrations should be preferred for long-term respiratory inflammation management as they are much less committed to systemic effects (Barchilon and Reinero 2023, Dowling 2024).

Regarding the **syndrome of equine asthma (SEA)**, treatment approach may also rely on corticosteroids, such as systemic dexamethasone or prednisolone, which are good for inflammatory reduction, while fluticasone is commonly used for inhalation strategies.

Bronchodilators like albuterol (inhaled) and clenbuterol (oral) are also useful resources. Administrations using a nebulizer system with mask like Flexineb® (Nortev Ltd, Galway, Ireland) have showed useful for inhaled administrations (de Wasseige et al. 2021).

For adjuvant approach in horse respiratory conditions, as individuals may be frequently subjected to airborne irritants, environmental management is commonly understood as mandatory. Hence, hay may be previously moistened to reduce dust-mites and molds influence, food should be pelleted, animals should be kept outside, and low-dust bedding should be preferred. Stalls should be washed frequently,

sweeping of the stable should be avoided when animals are inside, and stable ventilation should be improved.

The journal club will address 1-2 revision papers on skin barrier restoration and maintenance (*e.g.* Marsella 2025, Paixão et al. 2022).

At the end of the class, the therapeutic approach measures to allergic conditions in dogs, cats and horses will be summarized, in terms of providing well-being, by control of symptoms and prevention of complications, and by allergy triggering prevention through allergen avoidance, immune remodeling and skin barrier promotion.

Class 12

 # The allergen-specific immunotherapy and the multimodal treatment approach.

This class focuses on allergen-specific immunotherapy, the only patient-directed treatment option for allergy, with healing potential, by the induction of immune system remodeling (an allergen-reactive patient turns into an allergen-tolerant individual). Different types of ASIT and immune mechanisms associated will be assessed, clarifying different patterns of immune response, either successful or not.

Facing a diagnosis of allergy, like atopic dermatitis, therapeutic approach with systemic and topical medication, nutritional measures, ASIT, and environmental control may/should be equated in a multimodal perspective (Drechsler et al. 2024, Idée et al. 2022, Marsella et al. 2020, Popa et al. 2018, Cerrato et al. 2016, Olivry et al. 2015, Watson et al. 2006). It is also consensual that ASIT constitutes the only therapy with a healing potential, considering the pathophysiology of allergy. Furthermore, the most effective treatment may vary from patient to patient and even across the course of the disease in each patient (Olivry et al. 2015). A critical thinking perspective will outline the approach to multimodal treatment. Patient's diagnostic frame will be analyzed regarding susceptibility to allergen contact, their clinical consequences

and the possible ways to avoid the triggering process. Besides the avoidance measures equation, pharmacological prescription will bear in mind the patient (*e.g.* species, age and comorbidities), the environment and the species to which allergy occur, and the inherent foreseen treatment cost/benefit. Reasoning those factors will lead to a rational frame of treatment options to prescribe.

Aiming at the prescription of ASIT as effective as possible, in cases of environmental allergy, it is mandatory to identify the implicated allergen species by intradermal and serological testing (Pali-Schöll et al. 2020, Mueller et al. 2018, Martins et al. 2016). This process should be considered of high relevance because not much difference is usually found in sensitization between atopic and nonatopic dogs (Layne and DeBoer 2019). In addition, the immune system pathways may reveal difference regarding clinical manifestation between sensitized individuals, which may be related with the clinical relevance of IgE (Kumagai et al. 2021).

Regarding immune system remodeling, several changes have been reported in the course of ASIT in humans, such as the inhibition of dendritic cell (DC) maturation, the suppression of the release of inflammatory mediators, the inhibition of CD4+ T cell function, and the induction of isotype class switch from IgE to IgG and IgA (Olivry et al. 2015), the increase in Th1 and reduction in Th2 cells (Passalacqua and Canonica 2015, Loewenstein and Mueller 2009) or an increase of *in vitro* proliferation of PBMCs and of the relative proportion of Tregs, after allergen stimulation (Canonica et al. 2013).

An over-expression of IL-4 mRNA and a low transcription of TGF-β, was observed associated with atopic dermatitis in dogs, when compared to healthy skin. It has been also demonstrated that cAD was associated with an overproduction of IL-4, while healthy individuals' clinical tolerance was related with the expression of TGF-β. These observations were aligned with what had been observed in human patients subjected

to allergen-specific immunotherapy, where a reduction in IL-4 and an increase in IFN-γ, IL-10 and TGF-β was found associated with an isotype change from IgE to IgG (Nuttall et al. 2002). In fact, following ASIT in humans, the IgE/IgG imbalance is often changed, by decreasing IgE (Kindt et al. 2007, Jutel et al. 2003, Creticos et al. 1984, Lichtenstein et al. 1973) and increasing IgG (Kindt et al. 2007, Jutel et al. 2003, Lichtenstein et al. 1973).

In the course of ASIT, in animals, several immune changes occur such as inhibition of DC maturation, suppression of the release of inflammatory mediators, inhibition of CD4+ T cell function, and induction of an isotype class switch from IgE to IgG and IgA (Olivry et al. 2015).

In humans, an increase in Th1 and reduction in Th2 cells (Passalacqua and Canonica 2015, Loewenstein and Mueller 2009), or an increase of the *in vitro* proliferation of PBMCs and of the relative proportion of Tregs after allergen stimulation (Canonica et al. 2013) have been reported during ASIT. In this context, comparative studies between human and dog, regarding immune remodeling associated with ASIT, have been encouraged, considering dog as a feasible animal model for studies in human atopic dermatitis (Nuttall et al. 2002).

The key protective immunological mechanism, reason why healthy individuals do not develop allergies, seems to be the immune tolerance associated with Treg cells, as well as immunosuppressive cytokines associated with clinical improvement during ASIT. IL-10 secreting Tregs have been related to the control of allergen-derived immune response, by suppressing allergen-specific Th2-mediated IgE production and inducing IgG4 and IgA, as well as suppressing mast cells, basophils, and eosinophils (Loewenstein and Mueller 2009).

Testing of PBMCs from atopic dogs undergoing ASIT, previously stimulated with house-dust mite antigens, showed an increase in IFN-γ mRNA, with no change in IL-4 mRNA, resulting in a significant increase in the IFN-γ/IL-4 ratio, meaning a shift towards a Th1 response. By

assessing BALF from asthmatic cats under rush immunotherapy, a decrease in IL-4 and IL-5, and an increase of the transcription of IFN-γ and IL-10 mRNA was found. Evaluating Treg cells and IL-10 concentrations in healthy and atopic dogs undergoing ASIT, allowed to find an increase in both Treg and IL-10 concentrations, after favorable clinical evolution (Keppel et al. 2008).

Several main factors possibly affecting the clinical efficacy of ASIT in veterinary medicine have been pointed: i) patient selection; ii) allergen selection; iii) dose of allergens; iv) route of administration; v) vaccination schedule; vi) time to efficacy and compliance; vii) adverse reactions; viii) concomitant medication, and ix) re-evaluation during immunotherapy (Loewenstein and Mueller 2009).

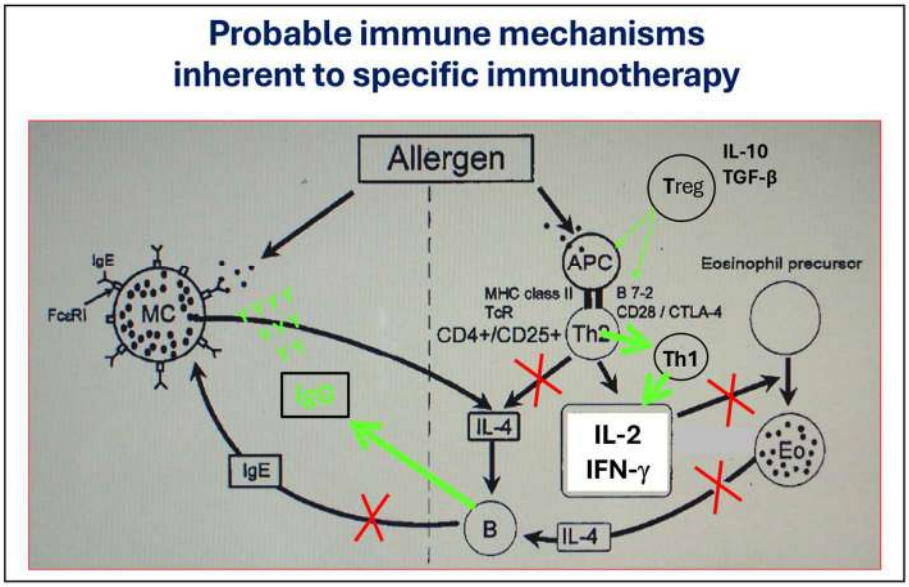
Despite poor responders having been found with an increase in both IgE and total IgG following ASIT, when compared to good responders (Loewenstein and Mueller 2009) successful ASIT was necessarily related to a significant increase in total IgG or IgG subclasses. In fact, different patients, all of them allergic to a given species, even presenting rather similar clinical conditions, may reveal differences in their individual sensitization allergograms, which mean different sensitization patterns to that species (Martins et al. 2017, Martins et al. 2015). However, when those patients undergo ASIT, the same extract from that species they have shown sensitized to, is commonly administered, despite their molecular genetic-based sensitization difference, which may lead to different individual success rates. So far, primary or cross-reactive sensitization is also not being thoroughly checked by classic diagnostic methods, which may also lead to possible immunotherapy flaws. Hence, it is useful to focus on the identification of the possible allergy-triggering molecular allergens as part of a CRD, which will allow identifying the more adequate pool of allergens for each individual as a tailor-made CRIT, aiming at a truly specific and therefore more effective ASIT (Canonica et al. 2013, Valenta et al. 1999).

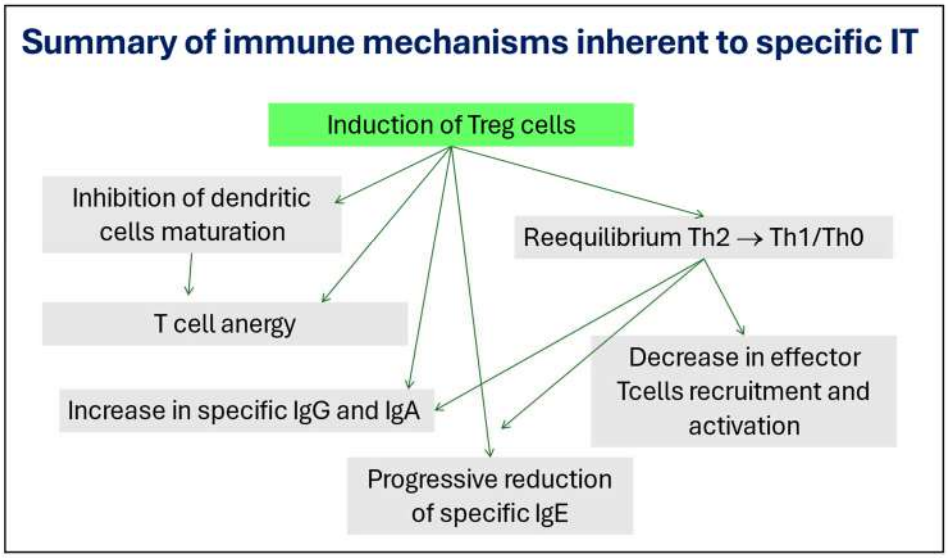
Regarding the administration of immunotherapy extracts, three main ways have been tested in animals: i) subcutaneous (or subcutaneous immunotherapy – SCIT); ii) sublingual (or sublingual immunotherapy – SLIT), and ILIT (or intralymphatic immunotherapy). Regarding epicutaneous (or epicutaneous immunotherapy – EPIT), there is a lack of data for animals. Different protocols, regarding doses and schedules have been recommended. In dogs, SCIT and SLIT are the most common ways of administration, and SCIT remains the main way of administration in current cat and horse ASIT. Regarding ILIT, it has been used in some allergic dogs not responding to conventional ASIT, but there is a lack of information with regard the usefulness of this way of administration in animals. At the end, strengths and weaknesses may be pointed to any of the administration ways (Mueller et al. 2018).

So far, in animals, ASIT has been considered for environmental allergy control, through the immune remodeling, aiming at beneficial clinical effect. Nevertheless, ASIT may not result in total healing as some allergy mechanisms may persist, leading to the need for other therapeutic support, even in lower doses or frequency of administration, which is clearly beneficial, considering the chronicity of the allergic condition. This is what has been considered the multimodal therapy approach as pointed by authors like Eisenschenk et al. (2024) and Olivry (2015). Finally, the control of the allergic condition will be better achieved through a multimodal therapeutic approach, using systemic and topical medication, nutritional measures, ASIT, and environmental control (Drechsler et al. 2024, Idée et al. 2022, Marsella et al. 2020, Popa et al. 2018, Cerrato et al. 2016, Watson et al. 2006). An update document, published in 2015 by Olivry et al. on behalf of the ICADA, and based on a 5-year period evaluation, recommended that treatment of cAD should be multimodal and “interventions should be combined for a proven (or likely) optimal benefit” (Olivry et al. 2015).

At the end of the class, a summary, highlighting the ASIT objectives, advantages and constraints within the frame of allergy pathophysiology and clinical manifestations will be produced. Students will be able to understand why ASIT is useful, however not the miracle cure for all allergies and patients, frequently needing the complementary support of supplemental therapeutic measures.

Examples of slides to be used in Lecture 12:





Class 13

 # Presentation and discussion of clinical cases by the students, based on their clinical activities

In this class, selected clinical cases, followed/being followed, will be presented (up to 10 min. each) by groups of four/five students, demonstrating the clinical reasoning in terms of the rational approach proposed in the discipline for the relevant facets of the allergic condition. The presentation of the clinical cases, including a summarized discussion, will be previously made available Clinical report should focus on:

- clinical history/anamnesis;
- clinical manifestations;
- complementary diagnostic tests (performed, not performed, and reasoning);
- tested treatment and results, further recommendations with specific reasoning, and
- overall evolution and further recommendations equated.

Teaching staff (jury of the discipline) will challenge the discussion regarding diagnosis and treatment options (up to 5 min/case) considering the clinical presentation and results of clinical evolution.

Class 14 (tutorial)

 # Summary of the matter-related objectives and student coaching

Conclusion of the presentation and discussion of clinical cases by the students may still occur in the first part of the class.

The matter objectives will be summarized regarding the following items, and discussed:

- hypersensitive mechanisms and allergy;

- diagnosis of allergic conditions such as atopy, asthma, insect-bite and contact allergy;
- identification of relevant implicated allergen species and allergens, through the available complementary diagnosis methods, in the context of precision medicine, and
- therapeutic options.

4.2.1. **Evaluation**

Evaluation will be composed of two main components such as a final written exam (70%) and the presentation and discussion of the selected clinical case (30%) by 4-5 students' groups, on the classes no. 13 and 14.

The written exam will be composed of questions covering the main matters addressed in the discipline. Questions will be mainly of multiple-choice and with spaces to fill. There are questions aiming to evaluate the knowledge on immune system, diagnosis paradigms and therapeutic options, and others evaluating the integrative capacity to formulate the best approach option for a given case.

An example of a written test is presented below:

4.2.1.1. **Example of an exam of Veterinary Immunoallergology**

Duration of 1.5h
Questions have equal score for a total of 20 points

1. Indicate one of the main characteristics of hypersensitivity reactions, in general:
   ___ Great severity.
   ___ Recurrence of complaints.
   ___ Caused by enzyme insufficiency.
   ___ Caused by immune activation.

2. How should the existence of a hypersensitivity reaction to substance X, of moderate intensity, be proven?
   ___ Provocation test with substance X
   ___ Skin tests with substance X.
   ___ Basophil activation tests with substance X.
   ___ Serum specific IgE assay for substance X.

3. Drug-induced hepatitis due to drug hypersensitivity is usually a hypersensitivity reaction of the following type:
___ Type II.
___ Type IV b.
___ Type IV c.
___ Type IV d.

4. Regarding the p-i concept in drug hypersensitivity, indicate the correct statement.
___ May explain reactions upon first administration of the drug.
___ May result from covalent binding of the drug to HLA/MHC molecules.
___ Implies reaction of the drug with pre-existing intracellular proteins.
___ Involves mast cell degranulation, induced directly by the drug, without the need for specific IgE.

5. The clinically relevant nature of IgE appears to be related to:
___ The variable component (paratope) of the IgE molecule.
___ The concentration of allergens for which specific IgE.
___ The affinity for mast cell receptors of the common component of the IgE molecule.
___ The existence of superantigens for which IgE has specificity.

6. Atopy can be understood as:
___ An individual or familial predisposition, genetically based, often hereditary, leading to the production of IgE with specificity for molecules that are often protein-based and leading to the development of symptoms associated with type I hypersensitivity.
___ An individual or familial tendency, genetically based, often hereditary, leading to the production of IgE with specificity for molecules that are often protein-based and leading to the development of symptoms associated with hypersensitivity reactions.
___ An individual or familial tendency, genetically based, often hereditary, leading to the development of immune activation against protein-based molecules, leading to the expression of allergic symptoms.
___ An individual or familial tendency, genetically based, often hereditary, leading to the production of low levels of IgE with specificity for molecules that are protein-based, associated with the expression of different types of hypersensitivity reactions.

7. In dogs, the diagnosis of atopy – yes or no – is based:
___ On a compatible past history and the determination of species-specific IgE levels for environmental allergens.
___ On a a compatible past history, in an exclusive work-up of divergent causes and in the result of verifying the Favrot criteria.
___ In the observation of frequent pruritus, in the face of evident allergy skin tests, positive for environmental allergens, and equally expressive specific IgE.
___ In a compatible past history and in an exclusive work-up of divergent causes, even if verifying the Favrot criteria is not very evident.

8. The practical usefulness of clinical evaluation methods such as CADESI-4 or SCORFAD:
___ Depends on the correct adherence of the owners to the clinicians' instructions.
___ Varies substantially with the clinician's evaluative criteria.
___ Does not depend on the therapy instituted.
___ Does not depend on the animal being simultaneously atopic and allergic to food.

9. Microarray or Western blot methods have diagnostic utility for:
___ The possibility of identifying allergic conditions when the clinical approach is negative.
___ The identification of food species implicated in allergy without digestive expression.
___ The possibility of substituting Traditional specific IgE panels.
___ The possibility of characterizing cross-reactivity phenomena between allergenic species, facilitating complementary diagnosis.

10. Equine atopy:
___ May present as dermatitis, but pruritus is not frequently associated.
___ Unlike in dogs, equine atopy does not appear to be particularly associated with skin barrier impairment.
___ It is a condition with possible pruritic skin manifestations, associated with type I hypersensitivity.
___ It is frequently associated with food allergy.

11. Equine atopic dermatitis:
___ Is predominantly associated with the inhalation of allergenic substances.
___ It is particularly associated with contact with allergenic substances.
___ It is suspected to be associated with the deep penetration of allergens into the skin, triggering a type I hypersensitivity response.
___ It is a frequent consequence of hypersensitivity to insect bites.

12. In equine immunoallergology, allergen-specific immunotherapy:
___ Has significant therapeutic utility in any type of allergy.
___ Has significant therapeutic utility after identification of associated sensitization.
___ It has not been shown to be viable via Sublingual.
___ It is equally useful in the clinical management of contact dermatitis.

13. Urticarial reactions in horses:
___ Are frequently associated with drug or insect bite allergy.
___ Are frequently associated with atopy or insect bite allergy.
___ Are very characteristic of nut allergy.
___ Are rare in drug allergy.

14. Superficial perivascular dermatitis:
___ Is the most common pattern observed in allergic diseases.
___ Is etiologically specific.
___ Indicates the agent's portal of entry into the dermis.
___ Is typical of autoimmune diseases.
___ Is always associated with vasculitis.

15. Which drugs should be discontinued before performing a skin biopsy?
___ Corticosteroids.
___ Antibiotics.
___ Cyclosporine.
___ Oclacitinib.
___ Antihistamines.

16. What precautions should be taken during a punch biopsy?
___ Do not include normal skin.
___ Use the smallest diameter to cause the least damage.
___ Include the transition in alopecic lesions.
___ Place it on a firm surface and let it rest on the table.
___ All of the above.

17. When performing a skin biopsy, it is important to:
___ Vigorously wash the skin to remove contaminants.
___ Perform surgical trichotomy to facilitate sample processing.
___ Remove crusts, as these are secondary lesions.
___ Select different evolutionary aspects of the lesions.
___ Do not send all the information, so as not to influence the diagnosis.

18. What type of hypersensitivity reaction characterizes feline asthma?
___ Type I.
___ Type II.
___ Type III.
___ Type IV.

19. Which of the following diseases, commonly misidentified as feline asthma, does NOT result in clinical symptoms?
___ Aelurostrongylosis.
___ Heartworm-associated respiratory disease.
___ Chronic bronchitis.
___ Toxocariasis.

20. Which of the following diseases, commonly misidentified as feline asthma, results in neutrophilic airway inflammation?
___ Aelurostrongylosis.
___ Heartworm-associated respiratory disease.
___ Chronic bronchitis.
___ Toxocariasis.

21. Which treatment for feline asthma, if used daily and long-term, will worsen airway inflammation?
___ Prednisolone.
___ Albuterol.
___ Cyclosporine.
___ Omega-3 fatty acids.

22. A horse presented to the hospital complaining of poor performance. Had presented an occasional dry cough for over a month, but body temperature was 37.4 °C. After a clinical examination, it was found that the animal did not present increased respiratory effort at rest.
It was considered that, presumptively, we could be dealing with a situation of _____, which according to the latest international consensus would be classified as type _____.
According to this consensus, in addition to bronchial endoscopy, what other complementary diagnostic method should be evaluated? _____.
What order of magnitude of value would you expect to find? _____.

23. A horse with a history consistent with severe asthma was presented to the hospital. After a clinical examination, it was considered that bronchoalveolar lavage was indicated.
What type of analysis should be performed on this lavage fluid? _____.
Which "value/element" would you expect to be abnormally high? _____.
Which "value/element" do you expect to be abnormally decreased? _____.

24. Which of the following oral corticosteroids is most effective in treating equine asthma?
___ Prednisolone.
___ Fluticasone.
___ Dexamethasone.
___ Betamethasone.

25. Why is the administration of bronchodilators, via aerosols, not indicated in horses with Equine Asthma Syndrome during an exacerbation phase?
_____
Which of the bronchodilators – clenbuterol, ipratropium, albuterol, and atropine – act as agonists at β2 receptors? _____, and which are antagonists of M receptors? _____.
Which of these can lead to paralytic ileus in horses? _____.

26. In the study presented on the incidence of allergic conjunctivitis associated with canine atopic dermatitis, the percentage of cases diagnosed by dermatology versus ophthalmology was:
___ 16% *vs* 60%.
___ 50% *vs* 50%.
___ 60% *vs* 16%.
___ None of the above.

27. The Conjunctival Provocation Test allows for the assessment of:
___ The severity of allergic conjunctivitis.
___ The etiology of allergic conjunctivitis.
___ The severity of keratitis.
___ The duration of exposure to allergens.

28. Allergy-associated conjunctivitis:
___ Is often follicular, associated with local accumulation of lymphocytes.
___ Commonly presents as pronounced chemosis.
___ Typically progresses to purulent conjunctivitis.
___ Frequently progresses to keratoconjunctivitis sicca.

29. What are the problems specifically associated with hypoallergenic diets?
___ Ingredient labeling/identification errors.
___ Cross-reactivity between animal proteins.
___ Generally, more expensive.
___ Current lack of completely allergen-free diets.
___ All of the above.

30. The most common gastrointestinal manifestation of canine food allergy is:
___ Vomiting.
___ Diarrhea.
___ Flatulence.
___ Frequent defecation.
___ Abdominal pain.

31. The most frequently allergenic food for dogs is
___ Rabbit.
___ Horse.
___ Fish.
___ Lamb.
___ Chicken.

32. The recommended test for diagnosing food allergy is:
___ Prick testing.
___ Patch testing.
___ Elimination diet.
___ Serology.
___ Intradermal.

33. The diagnosis of food allergy:
___ Is primarily based on the observation of digestive symptoms after the ingestion of suspected foods.
___ Is based on the improvement of the clinical condition, resulting from the implementation of exclusion diets, followed by symptomatic recurrence associated with food challenge tests.
___ Can be based on the detection of specific IgE to foods, such as beef, milk, or smoked foods.
___ Is based on the improvement of the clinical condition, subsequent to the ingestion of anti-inflammatory doses of corticosteroids.

34. Regarding Hypersensitivity to Insect Stings, which of the following statements is false.
___ It is characterized by the presence of pruritus and skin lesions with or without crusted papules.
___ Treatment may involve corticosteroid therapy.
___ The most frequently implicated agent is Culex (genus).
___ Contact with the implicated allergens should be eliminated.

35. Still regarding Insect Sting Hypersensitivity, which of the following statements is false?

___ The typical location of the lesions is at the base of the mane and tail, with other locations being possible.

___ Symptoms appear most frequently in horses from one year of age onwards and often worsen with age.

___ The severity and chronicity of the symptoms can lead to permanent skin lesions, with obvious aesthetic impairment.

___ Intense pruritus, in addition to potentiating secondary infections, is often associated with behavioral changes, which could lead to weight loss.

36. In contact allergy:

___ Since the dermatological manifestation appears only following contact, the role of the skin barrier is of little relevance.

___ The immunological mechanisms involved differ significantly from type I hypersensitivity.

___ History Previous history appears to be the only diagnostic route.

___ Hairy areas of the body are often the most affected, due to the retention of allergens in the fur.

37. In the diagnosis of contact allergy:

___ A history, identification of products/materials with which the animal comes into contact, and the results of avoidance are essential.

___ Intradermal testing is a very important diagnostic method for identifying the allergen source involved.

___ The least frequently affected areas are the glabrous areas, because the skin barrier is more effective there.

___ Evidence of provocation or contact should not be considered.

38. In the treatment of contact allergy:

___ Topical administration of corticosteroids should be avoided due to the possible development of skin atrophy.

___ Topical administration of immunomodulators (e.g., tracholimus) should be avoided due to the possible induced immunocompromise.

___ Topical administration of corticosteroids and immunomodulators is a rational option.

___ After therapeutic resolution, provocation tests should be performed to complete the diagnosis.

39. In the treatment of canine atopic dermatitis:

___ Diagnostic information associated with the identification of the agents to which the animal is sensitized and clinically reactive is of utmost importance, whether allergen-specific immunotherapy is chosen or not.

___ If allergen-specific immunotherapy is chosen, it is unnecessary to implement complementary therapeutic measures.

___ The possible development of anti-IgE or anti-IL-31 immunotherapy is an option to be followed, even if it lacks specificity regarding sources/species. Allergens.

___ Antihistamines are the preferred treatment, especially in chronic conditions, due to their lack of side effects, such as those associated with corticosteroids.

40. During allergen-specific immunotherapy, in cases of atopic dermatitis:

___ Corticosteroids may be indicated.

___ Skin barrier reestablishment therapy is very important.

___ Drugs such as oclacitinib or lokivetmab may be indicated, especially in the early stages.

___ Immunomodulators such as cyclosporine should be avoided.

___ All of the above are correct.

41. In the treatment of flea allergy:

___ A symptomatic approach should not be implemented, as it interferes with the results of parasite control.

___ A symptomatic approach is very important for various reasons, including avoiding infectious complications.

___ Antiparasitic treatment of the affected animal is essential.

___ Environmental antiparasitic control is essential.

42. In equine allergy to Culicoides:

___ It is important to apply insect repellents, place very fine-mesh screens on stable windows, and eliminate environmental conditions conducive to their development.

___ The administration of corticosteroids is a useful option and free of contraindications.

___ Specific subcutaneous immunotherapy has proven to be a clearly favorable option.

___ Specific sublingual immunotherapy has proven to be a clearly favorable option.

43. In the treatment of equine atopic dermatitis:
___ Allergen-specific immunotherapy is a less useful resource.
___ Allergen-specific immunotherapy is especially advantageous if it includes a broad range of allergenic species.
___ Allergen-specific immunotherapy is especially advantageous if it is based on the results of skin tests and circulating IgE panels.
___ Allergen-specific immunotherapy, administered sublingually, has proven to be ineffective.
___ None of the above options available are correct.

44. In the control of equine pruritus associated with atopic dermatitis:
___ The administration of oclacitinib has shown promise.
___ The administration of topicals such as corticosteroids and immunomodulators has shown little effectiveness.
___ The administration of antihistamines (e.g., hydroxyzine) may constitute a good therapeutic complement.
___ The administration of supplements containing essential fatty acids should be avoided due to the high risk of inducing food allergy.

45. The most prevalent pathogen in canine pyoderma is:
___ *Staphylococcus aureus*.
___ *Staphylococcus pseudintermedius*.
___ *Pseudomonas aeruginosa*.
___ *Proteus mirabilis*.

46. A 3-year-old male French Bulldog is presented to the clinic with papules, pustules, and crusts affecting the back and belly for 1 month. The attending physician suspects bacterial folliculitis. The patient has been healthy and, to date, has never had any skin problems. The first diagnostic method to be proposed to the owner will be:
___ Skin biopsy.
___ Aerobic bacterial culture.
___ Skin cytology.
___ Allergy testing.

47. Regarding the previous case, the Veterinarian decides to recommend antibiotic therapy. The most appropriate option is:
___ Metronidazole.
___ Clindamycin.
___ Enrofloxacin.
___ Gentamicin.

48. Recurrences of bacterial folliculitis are associated with a primary cause. The most common primary cause found in dogs is:
___ Hypothyroidism.
___ Demodecosis.
___ Atopic dermatitis.
___ Autoimmune diseases.

5. Bibliography

References are divided into essential and complementary, the first part representing what is considered fundamental for the understanding of the concepts, and the second part what would be considered an advanced complement to provide an in-depth understanding of the matters. These latter references are particularly targeted at students who may manifest further interest in immunoallergology or, like the professionals, have the prospect of developing work in this area.

Both essential and complementary bibliography will be made available through the MOODLE platform, the sponsored university platform (b-on) or free access.

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