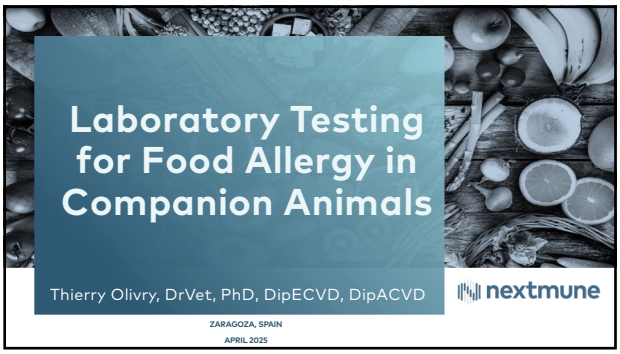


1

Disclosure

I am the Head of R&D
at
nextmune

Driven by Science
Powered by Passion

2

CAT 4: diagnostic tests for food allergies

RESEARCH ARTICLE Open Access

Critically appraised topic on adverse food reactions of companion animals (4): can we diagnose adverse food reactions in dogs and cats with in vivo or in vitro tests?

Ralf S. Mueller[1*] and Thierry Olivry[2]

(Mueller and Olivry, BMC Vet Research 2017)

3

CAT 4: diagnostic tests for food allergies

	Accuracy	Positive predictability	Negative predictability	
Intradermal testing with food antigens [4, 14]	63–76%	60–67%	62–77%	IgE-mediated allergy
Serum testing for food-specific IgE [4, 5, 14]	58–87%	15–100%	61–86%	
Serum testing for food-specific IgG [5]	77%	35%	84%	
Lymphocyte proliferation tests [14]	94%	100%	93%	cell-mediated allergy
Patch testing with food antigens [5, 25]	81–90%	63–75%	88–99%	

The accuracy was calculated by dividing the number of correct results by the number of all results verified, positive predictability by dividing correctly positive results by the total number of positive results and negative predictability by dividing correctly negative results by the total number of negative results

(Mueller and Olivry, BMC Vet Research 2017)

4

CAT 4: diagnostic tests for food allergies

	Accuracy	Positive predictability	Negative predictability	
Serum testing for food-specific IgE [15]	20%	0%	20%	IgE-mediated allergy
Lymphocyte proliferation tests [15]	80%	100%	50%	cell-mediated allergy
Intradermal tests [15]	47%	100%	27%	

The accuracy was calculated by dividing the number of correct results by the number of all results verified, positive predictability by dividing correctly positive results by the total number of positive results and negative predictability by dividing correctly negative results by the total number of negative results

(Mueller and Olivry, BMC Vet Research 2017)

5

CAT 4: diagnostic tests for food allergies

RESEARCH ARTICLE Open Access

Critically appraised topic on adverse food reactions of companion animals (4): can we diagnose adverse food reactions in dogs and cats with in vivo or in vitro tests?

"Testing for serum food-specific IgE and IgG showed low repeatability and, in dogs, a highly variable accuracy [for the diagnosis of adverse food reactions]. In cats, the accuracy of testing for food-specific IgE was low..."

"Currently, the best diagnostic procedure to identify adverse food reactions in small animals remains an elimination diet with subsequent provocation trials"

(Mueller and Olivry, BMC Vet Research 2017)

6

What is food allergy?

GA²LEN guidelines 2022: *An **adverse reaction to food** mediated by an **immunologic mechanism**, involving **specific IgE** (IgE-mediated), **cell-mediated mechanisms** (non-IgE-mediated), **or both IgE- and cell-mediated mechanisms** (mixed IgE- and non-IgE-mediated)*

(Muraro, World Allergy Organization J 2022)

7

IgE-mediated food allergy: clinical diagnoses

- urticaria, angioedema
- anaphylaxis
- oral allergy syndrome (pollen-food syndrome)
- rhinitis
- asthma
- immediate gastro-intestinal hypersensitivity
- delayed food anaphylaxis to mammal meat (α-gal syndrome)
- food-associated, exercise-induced anaphylaxis

time to flare after ingestion:
→ *most common: within 2 hours*
→ *less common: 2-6 hours*

(Sicherer, J Allergy Clin Immunol 2018)

8

Non-IgE-mediated food allergies: clinical diagnoses

- allergic contact dermatitis
- food protein-induced enterocolitis syndrome (FPIES)
- food protein-induced allergic proctocolitis
- food protein-induced enteropathy
- dermatitis herpetiformis
- Heiner syndrome
- celiac disease
- fixed food eruption

time to flare after ingestion:
→ *more than 4 hours*

(Sicherer, J Allergy Clin Immunol 2018)

9

Mixed IgE/non-IgE food allergies: clinical diagnoses

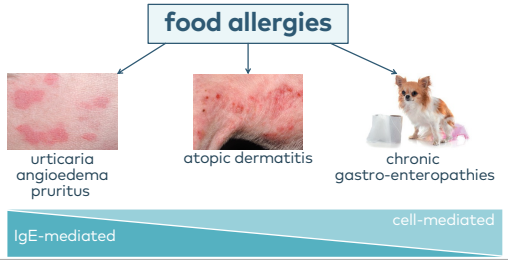
- atopic dermatitis
- eosinophilic oesophagitis
- eosinophilic gastro-enteropathies

time to flare after ingestion:
→ *variable*
→ *from 6 to 48 hours*

(Sicherer, J Allergy Clin Immunol 2018)

10

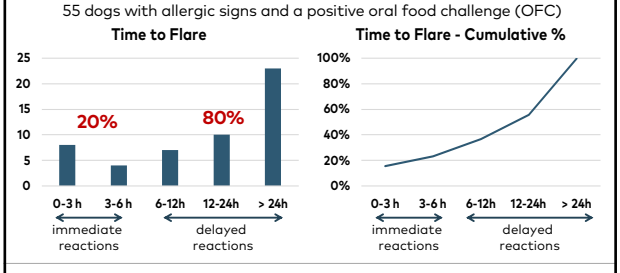
What is food allergy?



11

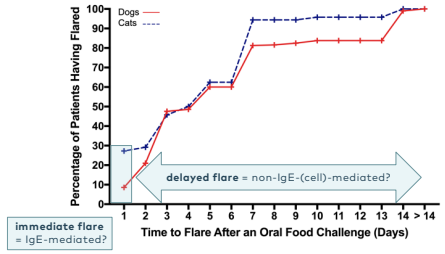
Dogs with food allergy: 2022-2023

55 dogs with allergic signs and a positive oral food challenge (OFC)



12

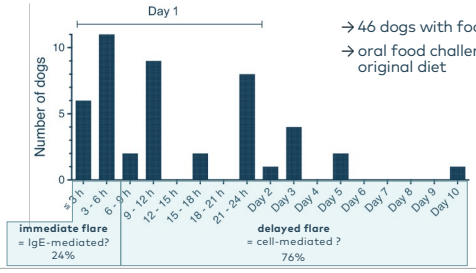
Time to flare after oral food challenge



(Olivry and Mueller, BMC Vet Research 2020)

13

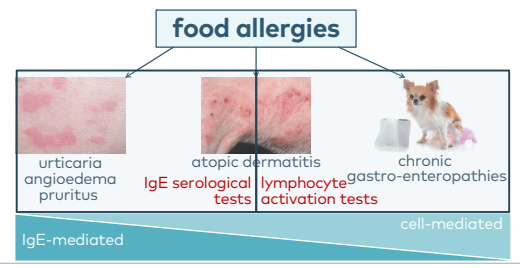
Time-to-flare after oral food challenge



(Shimakura, Vet Dermatol 2021)

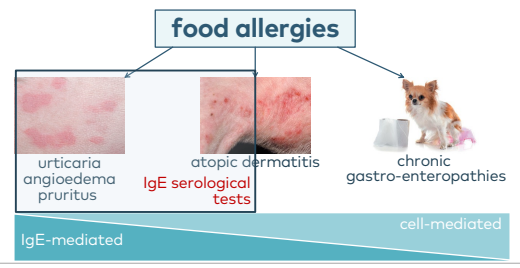
14

Sensitization tests for food allergy



15

Sensitization tests for food allergy



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Food allergen-specific IgE serology

Gadus macrocephalus

- dogs with AD: 179
- positive cod challenge: 27/179 (15%)
  - **signs < 12 hrs: 18/27 (67%)**
  - signs > 12 hrs: 9/27 (33%)
- positive cod sIgE: 12/27 (44%)
  - **signs < 12 hrs: 12/18 (67%)**
  - signs > 12 hrs: 0/9 (0%)

(Imanishi, BMC Vet Research 2020)

Gallus gallus domesticus

- positive food/chicken challenge: 12
  - **signs < 12 hrs: 2/12 (17%)**
  - signs > 12 hrs: 7/12 (58%)
  - unknown 3/12 (25%)
- positive chicken sIgE: 7/12 (58%)
  - **signs < 12 hrs: 2/2 (100%)**
  - signs > 12 hrs: 2/7 (29%)
  - unknown 3/3 (100%)

(Olivry, Vet Dermatol 2022)

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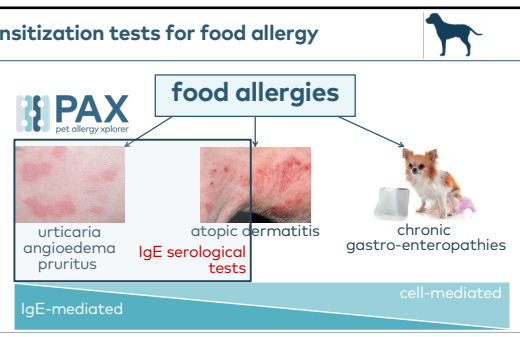
Sensitization tests for food allergy



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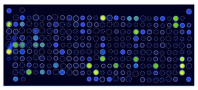
Nextmune PAX with MADX technology

PAX pet allergy xplorer

- automated **quantitative multiplex macroarray** with cartridges containing up to 300 allergen-covered nanoparticles:
  - one-third of "classical" extracts
  - two-thirds of molecular components
- **dogs:** January 2023
- **horses:** September 2023
- **cats:** January 2024



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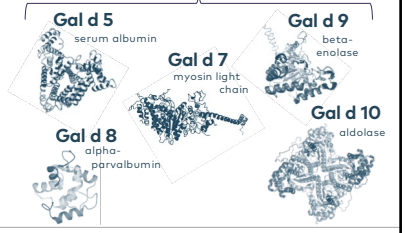
Molecular allergology

EXTRACT | MOLECULAR COMPONENTS

Gallus domesticus



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Food allergy case 1

- Labrador, female, 3-year-old
- 2-year history of recurrent diarrhea
- controlled on RC gastrointestinal (chicken, eggs, corn, rice)
- changed to Purina HA (hydrolyzed soy and corn)
- flare of signs **within 1 hour**
  - **bloody diarrhea**
  - **erythema, conjunctivitis**

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Food allergy case 1

- change of diet to Hill's i/d (eggs, corn, rice, barley, oats)
- rapid improvement of diarrhea
- resolution of all signs within 2 weeks

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Food allergy case 1

PAX pet allergy xplorer

	ng/mL	class	
• Gly m 5:	33	1	soybean 7S vicilin
• Ves v 5:	29	1	

→ provocation with soy = **relapse of signs within 3 hours**

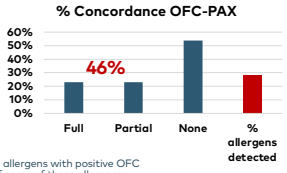
→ ingestion of one hazelnut = **vomiting and pruritus in 30 mins**

= cross-reactivity between Gly m 5 and Cor a 11 or Cor a 16 7S vicilins?

29

Dogs with immediate reactions (0-6h post OFC)

55 dogs with allergic signs and a positive oral food challenge (OFC)
10 dogs with immediate reactions



PAX pet allergy xplorer

Full: identification of all allergens with positive OFC
Partial: identification of some of these allergens

limited performance of the PAX to detect IgE sensitizations in dogs with immediate food allergies

30

Dogs with immediate reactions (0-6h post OFC)

55 dogs with allergic signs and a positive oral food challenge (OFC)
10 dogs with immediate reactions

% Concordance OFC-PAX

60%
50%

PAX pet allergy xplorer

IMPORTANT

LIKELY UNDERESTIMATION OF CONCORDANCE OF PAX-OFC BECAUSE OF LITTLE OFC DATA

allergens detected

Full: identification of all allergens with positive OFC
Partial: identification of some of these allergens

limited performance of the PAX to detect IgE sensitizations in dogs with immediate food allergies

31

Immunoblotting + mass spectrometry in dogs with immediate food reactions and negative PAX food

carbonic anhydrase
adenylate kinase *
triosephosphate isomerase
phosphoglycerate mutase *
myomesin 2 *
troponin C
Tri a 17 (beta-amylase)
Zea m globulin-2
Ave s 12S seed storage protein *

* not described as an allergen before!

All but one of these components are unknown allergens for these foods in dogs! (some are not even reported allergens for humans!)

32

Molecular Indexing of Proteins by Self-Assembly (MIPSA)

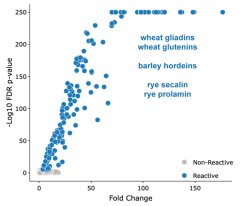
INFINITY BIO
AllerSIGHT™: allergy reactome

4,463 protein sequences
2,352 distinct allergens

N C

~ 30 000, DNA-barcoded, 56-amino acid long peptides overlapping over 28 amino acids

1. IgE immunoprecipitation of library
2. PCR amplification of barcodes
3. DNA sequencing and counting



(Credle,Nature Biomed Engineering 2022)

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Sensitization tests for food allergy

food allergies

urticaria angioedema pruritus
atopic dermatitis
chronic gastro-enteropathies
lymphocyte activation tests

IgE-mediated
cell-mediated

35

Lymphocyte proliferation test (LPT)

EDTA
centrifuge
add separation medium
plasma
mononuclear cells (PBMCs) medium + neutrophils
red blood cells
automatic cell counter
add food extracts
count cells (pre)
count cells after five days (post)

stimulation index (SI)
= allergen:saline (post-stimulation, Day 5)

(Fernandez-Losano, Vet Dermatol, in press 2025)

36

Threshold (cut-off) determination

- 10 laboratory beagles, 4.5-6.5 years, all females
- same chicken-and-rice diet for the last 3 years
- LPT with 23 food extracts + 1 component (Bos d 7)

→ SI: 1.0 – 2.7 (median: 2.0; 95% CI: 1.9-2.0)
→ SI < 2.7 for all food ingredients on the label

☞ **threshold: mean + 3SD = 3.0 (240 values)**

(Fernandez-Losano, Vet Dermatol, in press 2025)

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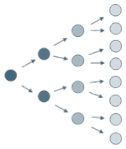
LPT in healthy pet dogs

- 25 healthy pet dogs with no signs of allergy:
  - → multiple breeds: 3 whippets
  - → median age: 7.5 years (2.0-16.0)
  - → female:male ratio: 0.9
- LPT with 28 food extracts + 1 component (Bos d 7)
  - → SI: 0.8 – 2.9 (median: 1.7) (725 values)

☞ **LPT specificity = 100%**



(Fernandez-Losano, Vet Dermatol, in press 2025)

38

Dogs with delayed food allergies and TTF ≥ 6 h

- signalment: 28 dogs
  - → multiple breeds: 4 retrievers
  - → median age: 3.0 years (0.5-12.0)
  - → female:male ratio: 0.8
- clinical signs:
  - → food-induced atopic dermatitis: 64%
  - → gastro-enteropathy: 64%
  - → pruritus without visible lesions: 18%

(Fernandez-Losano, Vet Dermatol, in press 2025)

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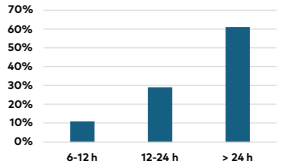
Time to flare after oral food challenge



Dog 6
- → chicken: 0.5 h
- → pork: > 24 h

Dog 11
- → beef: < 1 h
- → wheat: 1-3 h
- → chicken: > 24 h

Dog 12
- → pork: 1-6 h
- → others: 12-24 h

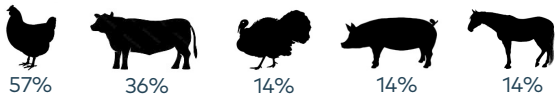
Dog 14
- → chicken, lamb, beef: 3-6 h
- → horse: 12-24 h

mixed food allergy?

(Fernandez-Losano, Vet Dermatol, in press 2025)

40

Top 5 food sources causing positive OFC ≥ 6 hours



(Fernandez-Losano, Vet Dermatol, in press 2025)

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LPT in dogs with delayed food allergies

- 28 dogs with delayed food allergies (TTF ≥ 6 h post-OFC)
- LPT with 13-29 food extracts + 1 component (Bos d 7) (median: 27)
  - → all dogs had at least 1 positive LPT with SI > 3.0
  - → number of food-positive LPT: 2-10 (median: 5)
  - → SI of positives: 3.0-10.2 (median: 3.7) (61 values)

☞ **LPT sensitivity = 100% for the identification of delayed food allergies in dogs**

(Fernandez-Losano, Vet Dermatol, in press 2025)

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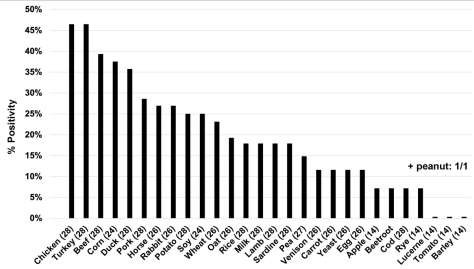
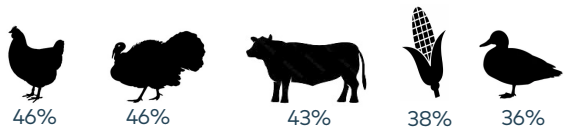
Food ingredients positive by LPT



(Fernandez-Losano, Vet Dermatol, in press 2025)

43

Top 5 food extracts positive by LPT



(Fernandez-Losano, Vet Dermatol, in press 2025)

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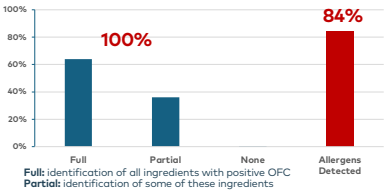
Concordance between LPT and delayed OFC (≥ 6 h)

28 dogs with delayed food allergy

PAX pet allergy xplorer
negative in 64% of these cases



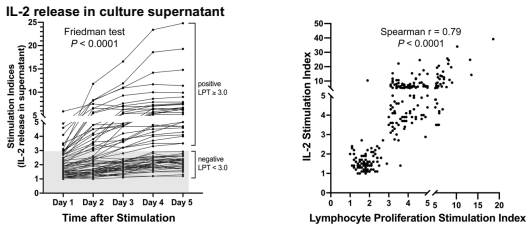
Full: identification of all ingredients with positive OFC
Partial: identification of some of these ingredients

→ identification of all ingredients in 18 dogs (64%); of some in 10 dogs (36%)
→ identification of 57/68 (84%) food ingredients with positive delayed OFC

(Fernandez-Losano, Vet Dermatol, in press 2025)

45

Lymphocyte proliferation versus IL-2 secretion



excellent correlation between cell counts and IL-2 release
= proliferating cells are most likely T cells

(Fernandez-Losano, Vet Dermatol, in press 2025)

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Lymphocyte proliferation test (LPT)

ORIGINAL ARTICLE

Veterinary Dermatology

Evaluation of a direct lymphocyte proliferation test for the diagnosis of canine food allergies with delayed reactions after oral food challenge

Carlos Fernandez-Lozano[1] | Ana Mas-Fontao[1] | Silvia T. Auxilia[2] | Marie Welters[2] | Alla Olivri[3] | Ralf S. Mueller[4] | Thierry Olivry[5]

(Fernandez-Losano, Vet Dermatol, in press 2025)

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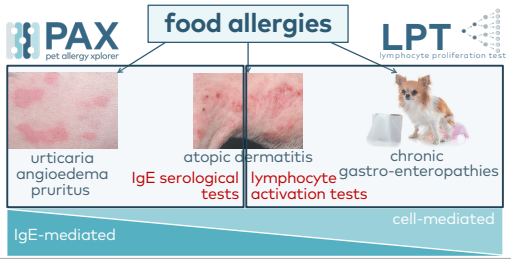
Sensitization tests for food allergy



48

Food allergy case 2

3-year-old, M, Staffordshire terrier with urticaria
→ **time-to-flare**: within 1 hour of eating a ham sandwich and also beef

PAX pet allergy xplorer
Bos d 6 (BSA)

not done

immunoblotting and mass spectrometry: IgE targeting pork serum albumin, triosephosphate isomerase (TPI1), adenylate kinase (ADK), pyruvate kinase (PKM)

dog with IgE-mediated (immediate) allergy to beef and pork

49

Food allergy case 3

12-year-old, F, Staffordshire crossbred dog with recurrent diarrhea after eating beef
→ **time-to-flare**: > 48h after oral food challenge

PAX pet allergy xplorer
no detectable food sensitizations

beef
lamb

dog with cell-mediated (delayed) allergy to beef and lamb (and other meats?)

50

Food allergy case 4

1-year-old, M, Siamese cat with facial, head, and neck pruritus, after eating fish (cod, sardines, and salmon)
→ **time-to-flare**: > 24h after oral food challenge

PAX pet allergy xplorer
no detectable food sensitizations

sardine
cod
rice*,
rye*
potato*
barley*

* no OFC data

cat with cell-mediated (delayed) allergy to fish (and others?)

51

Food allergy case 5

6-year-old FS, Spanish Alano with pruritus and diarrhea after eating yogurt or milk
→**time-to-flare**: > 24h after oral food challenge

PAX pet allergy xplorer

Bos d 6 (BSA)
Bos d 7 (IgG)
Bos d 8 (caseins)
Ovi a_meat
Equ c meat
Ory c GAPDH

LPT lymphocyte proliferation test

Bos d meat
Bos d 7 (IgG)
Bos d milk
Sus d meat

dog with mixed delayed allergy to dairy (and to other mammal meats?)
Could immediate signs of food allergy have been missed?

52

Food allergy case 6

4-year-old, female, French bulldog with a history of post-vaccine angioedema and urticaria after eating chicken, and a lamb-and-rice and fish-and-potato diets
→ **time-to-flare**: between 30 mins and 3 hours after oral food challenge and vaccination

PAX pet allergy xplorer

Bos d 6 (BSA)
Bos d 7 (IgG)
Ovi a_meat
Ovi a_IgG

Gal d (chicken)
Gad m (cod)
Ovi a (lamb)
Beta v (beet)*
Ave s (oat)*

* no OFC data

dog with IgE-mediated/mixed allergy to beef/lamb, chicken, fish (and others?)

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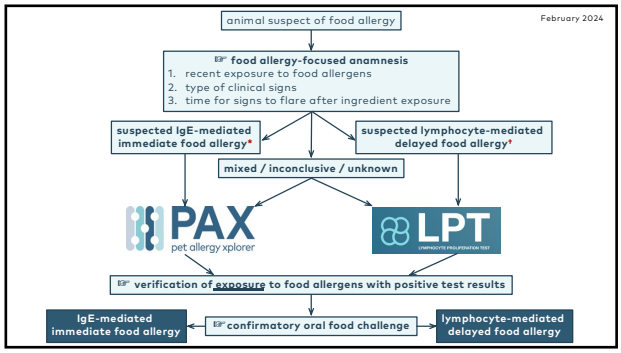
IgE-mediated food allergy: first steps of diagnosis

recommendation #1
In patients with suspected IgE-mediated food allergy, a ***detailed allergy-focused clinical history is recommended*** *as the first step of the diagnostic work-up*
(low certainty of evidence, expert opinion)

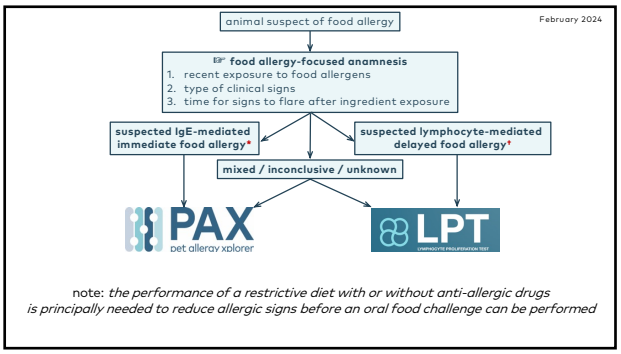
recommendation #2
In patients with a history of suspected IgE-mediated food allergy, ***skin prick test*** *and/or* ***measurement of serum specific IgE*** *are recommended as first-line tests in the diagnostic work-up for food allergy*
(high certainty of evidence)

(Santos, EAACI guidelines on IgE-mediated food allergy, Allergy 2023)

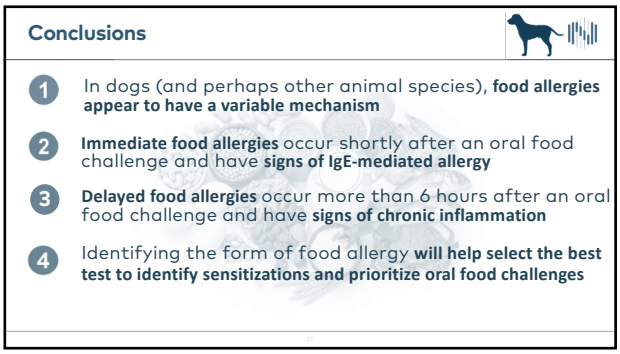
54



55


animal suspect of food allergy
February 2024
☞ food allergy-focused anamnesis
1. recent exposure to food allergens
2. type of clinical signs
3. time for signs to flare after ingredient exposure
suspected IgE-mediated immediate food allergy*
suspected lymphocyte-mediated delayed food allergy*
mixed / inconclusive / unknown
PAX
pet allergy xplorer
LPT
note: the performance of a restrictive diet with or without anti-allergic drugs
is principally needed to reduce allergic signs before an oral food challenge can be performed

56


Conclusions
1 In dogs (and perhaps other animal species), food allergies appear to have a variable mechanism
2 Immediate food allergies occur shortly after an oral food challenge and have signs of IgE-mediated allergy
3 Delayed food allergies occur more than 6 hours after an oral food challenge and have signs of chronic inflammation
4 Identifying the form of food allergy will help select the best test to identify sensitizations and prioritize oral food challenges

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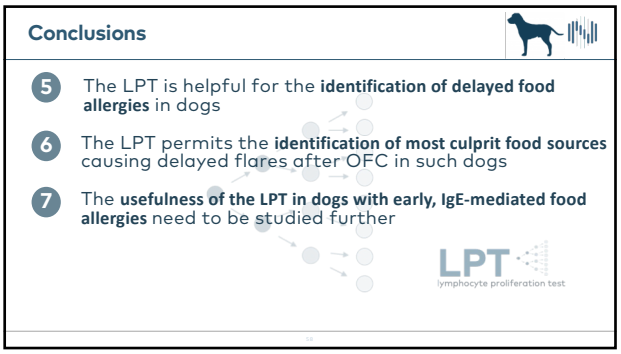
Conclusions
5 The LPT is helpful for the identification of delayed food allergies in dogs
6 The LPT permits the identification of most culprit food sources causing delayed flares after OFC in such dogs
7 The usefulness of the LPT in dogs with early, IgE-mediated food allergies need to be studied further
LPT
lymphocyte proliferation test

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