

Serum IgE cross-reactivity between fish and chicken meats in dogs

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Background – In humans, a cross-reactive clinical allergy has been reported between three chicken and fish meat proteins: beta-enolase, aldolase A and parvalbumin.

Objective – To evaluate if IgE cross-reactivity between chicken and fish also existed in the dog.

Animals – Sera from dogs with suspected allergic skin disease and with IgE against chicken and fish.

Methods and materials – Sera were analysed by ELISA and immunoblotting with chicken, white fish (haddock and cod) and salmon extracts. Reciprocal inhibition ELISAs and inhibition immunoblots were then performed. Protein sequencing of bands identified on multiple extracts was determined by mass spectrometry.

Results – Out of 53 archived canine sera tested by ELISA against chicken, white fish or salmon, 15 (28%), 12 (23%) and 26 (49%), respectively, had elevated IgE against one, two or all three of these extracts. Seven of the triple-reactive sera were subjected to reciprocal inhibition ELISAs. A >50% inhibition was found between chicken–fish, chicken–salmon and fish–salmon in seven, four and five of seven dogs, respectively. Immunoblotting identified multiple IgE-binding proteins of identical molecular weights in the three extracts; these were partially to fully cross-reactive by inhibition immunoblotting. Mass spectrometry identified nine cross-reactive proteins as: pyruvate kinase, creatine kinase, alpha-actin, glyceraldehyde-3-phosphate dehydrogenase, beta-enolase, aldolase, malate dehydrogenase, lactate dehydrogenase and triose-phosphate isomerase 1. All of these have been reported previously as fish, shellfish and/or chicken allergens for humans.

Conclusions and clinical importance – Whether any of these newly identified IgE cross-reactive chicken–fish allergens is the cause of clinical allergy needs to be determined in dogs reacting to at least two of these common food sources.

Introduction

In humans and animals, adverse food reactions (AFRs) are separated into either immunological food reactions (i.e. the food allergies) and the so-called food intolerances, whose mechanism does not involve the immune system. Although cutaneous AFRs are rather common in pruritic dogs and in those diagnosed with allergic or atopic dermatitis,¹ there is not yet a single case report documenting the occurrence of a nonimmune food intolerance in dogs. As a result, the vast majority of idiosyncratic cutaneous AFRs are likely to represent bona fide immunological food allergies due to a combination of IgE-^{2,3} and

lymphocyte-mediated reactions against dietary allergens.⁴

Cross-reactivity between food allergens is the subject of increasing interest. The presence of immunologically related allergens in different food sources could have important implications for the diagnosis and risk management of IgE-mediated food allergy in dogs. Sensitization to one commonly ingested food could result in acute clinical flares after eating other seemingly novel foods containing the same or similar IgE-binding epitopes. Hence, a food allergy could be erroneously ruled out upon failure to respond to a single or even multiple commercial or home-made diet trials, if the food items chosen for the elimination diet were to contain cross-reacting allergens. The taxonomic relationship between food sources could be an important consideration when selecting diets for a restriction trial. IgE serological cross-reactivity between related mammalian foods has been reported in dogs sensitized to beef, lamb and cow's milk.⁵ In a previous report, IgG and muscle phosphoglucosyltransferase were identified as major cross-reactive allergens in dogs with food allergy that had detectable serum allergen-specific IgE to beef, lamb and cow's milk.⁶ Bovine serum albumin (Bos d 6) also has been reported to be an allergen in beef-allergic

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dogs⁷ and, given its abundance in cow's milk,⁸ this evolutionarily conserved serum albumin could be an additional source of cross-reactivity between these two foods.

The conservation of proteins during evolution is an important determinant as to whether cross-reactivity is limited to closely related species or encompasses more taxonomically distant species. In humans, IgE-mediated cross-reactivity between homologous allergens from unrelated species could account for clinical syndromes such as pollen-associated food allergies.⁹ A number of studies have demonstrated the existence of shared IgE-binding proteins in unrelated foods.^{10,11} Most recently, an unexpected association between fish and chicken was noted, with 30% of chicken-allergic patients also found to be allergic to fish.¹² Suffering from the 'fish-chicken syndrome', patients with concomitant fish and chicken allergy react to homologous proteins present in both meats.¹³ Chicken parvalbumin (Gal d 8), aldolase A (Gal d 10) and (beta)-enolase (Gal d 9) have been recognized by specific IgE in 69%, 72% and 83% of 29 patients with concomitant fish and chicken allergy, respectively.¹³ Specific IgE reactivity to the homologous codfish proteins, parvalbumin (Gad m 1), enolase (Gad m 2) and aldolase (Gad m 3), also was shown in 52%, 90% and 66% of the same patients, respectively.¹³ Competitive IgE inhibition enzyme-linked immunosorbent assays (ELISAs) confirmed these allergen homologues to be highly cross-reactive and, thus, likely responsible for the clinical cross-reactivity between fish and chicken meat.^{13,14} Other recognized cross-reactive clinical syndromes are due to the above-mentioned serum albumin. Albumins are implicated not only in the expected cross-reactivity among mammalian meats, but also in the so-called "pork-cat"¹⁵ and "bird-egg"¹⁶ syndromes whereby the inhalation of cat/dog danders or bird feathers, respectively, results in sensitization and an ensuing clinical reactivity after eating insufficiently cooked meats or egg yolks, serum albumin being a thermolabile allergen. This allergen also causes clinical cross-reactivity between chicken and pork meats, especially if insufficiently cooked.¹⁷

The extent of immunological cross-reactivity among foods has not been well-studied in dogs. Consequently, it is not clear if the shared allergenic proteins associated with clinical syndromes in humans are pertinent to dogs. Chicken and fish are frequently and widely consumed by dogs, and are known allergic ingredients,¹⁸ but little is known of the specific proteins that are involved in IgE-sensitization to these foods. A significant positive association with specific IgE between white fish and chicken has been reported previously,⁵ suggesting that, as in humans, cross-reactivity between fish and chicken also could exist in dogs. The nature of this association was not, however, further investigated. The aim of this study was to determine if cross-reactive canine serum IgE-binding proteins could be identified in chicken, white fish and salmon.

Methods and materials

Canine sera

Surplus dog sera submitted to Avacta Animal Health for allergen-specific IgE serological testing were used for this study. Consent for use

of the sera in research was obtained by means of a tick-box in the corresponding submission forms. All sera had been stored at -20°C prior to use.

Extracts

The three food allergen extracts, chicken (C, *Gallus gallus domesticus*), white fish [WF, a mixture of the Gadidae-family Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) in equal protein concentration] and Atlantic salmon (S, *Salmo salar*) were all obtained from Greer Laboratories (Lenoir, NC, USA). The protein concentration of each extract was determined by measurement at 280 nm before use (Versa Wave, Expedeon; Cambridge, UK).

ELISA

We began with the analysis of results of 1,500 consecutive sera of dogs with suspected allergic disease submitted to the laboratory between October 2017 and February 2018 to determine the level of their IgE sensitization to C, WF and S. Chicken-, WF- and S-specific IgE ELISAs were then performed, as described previously,¹⁹ using 53 dogs that had been shown to have IgE directed against at least one of these three food allergens. Briefly, sera were diluted 10-fold in Tris Buffered Saline (TBS) containing 0.05% Tween 20 (TBST) before being added to extract-coated wells (5 $\mu\text{g}/\text{mL}$). Specific IgE was detected using alkaline phosphatase (AP)-labelled anti-dog IgE (clone 5.91, North Carolina State University; Raleigh, NC, USA) and AP substrate (pNPP, BioRx Laboratories; Owings Mills, MD, USA). Serial dilutions of a standard reference serum, comprising a pool of dog sera with high levels of allergen-specific IgE, were used to construct a standard curve. Serum IgE reactivity was quantified into arbitrary units of antibody (AU) as determined from absorbance values (OD405) of test sera interpolated from the resultant standard curves. The assay cut-off was determined using the mean absorbance plus three times the standard deviation of a large number (94 sera) of *in vitro* C, WF and S IgE-negative samples, as determined by previous ELISAs.

Inhibition ELISA

Inhibition ELISAs were performed to ascertain if co-positive IgE reactions were the result of immunological cross-reactivity or simple co-sensitizations. Seven dogs that were co-positive to C, WF and S allergen extracts, negative to potato extract, and had a volume in excess of 400 μL were selected for this part of the study. To address the possibility that the addition of inhibitor solution could positively affect the assay signal, one dog that had previously been shown to have no IgE reactivity to the extracts also was assayed. Sera were diluted 10-fold in TBST both with and without 1,000 $\mu\text{g}/\text{mL}$ C, WF, S allergen extract inhibitors. For negative control inhibition, sera were pre-incubated with 1,000 $\mu\text{g}/\text{mL}$ potato allergen extract. All serum-inhibitor mixes and no-inhibitor controls were incubated overnight at $2-8^{\circ}\text{C}$ before analysis by ELISA (as described previously). The percentage of inhibition was calculated according to the following formula: inhibition (%) = $100 - [(\text{OD405 of serum with inhibitor} / \text{OD405 of serum without inhibitor}) \times 100]$, where OD405 was the absorbance at 405 nm.

The concentration of inhibitor was determined from a preliminary experiment using six dog sera, which showed that a concentration of 1,000 $\mu\text{g}/\text{mL}$ of C extract was required for the maximum inhibition (>84%) of IgE-binding to the homologous coats. At this concentration, there was minimal inhibition (5%) by the negative (potato) inhibitor.

SDS-PAGE of extracts

Denaturing SDS-PAGE was performed to separate extract proteins. Briefly, extracts were heated at 70°C for 10 min in the presence of a reducing agent (Expedeon) before electrophoresis. Protein extracts (2 μg per lane) were separated on RunBlue 4%–12% Bis-Tris precast gels (Expedeon) at 180 V in Tris-MES-SDS running buffer on a Run-Blue Dual Run & Blot Unit (Expedeon). Molecular weight markers (180–5 kDa; RunBlue Tricolour markers, Expedeon) were run in parallel to the extracts. Following electrophoresis, the gels were either stained using InstantBlue Coomassie stain (Expedeon) to visualize

protein components, or proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Expediton) for immunoblotting.

Inhibition immunoblotting

Sera from three dogs with high C-, WF- and S-specific IgE signal ($>OD_{405}$: 0.5, by previous ELISAs) were pooled. A second pool comprising sera from five dogs with no detectable serum IgE to C, WF or S ($<OD_{405}$: 0.2, by previous ELISAs) was prepared as a negative control. Blotting was performed at 200 V for 1.5 h in Tris/glycine transfer buffer (Expediton) containing 10% methanol using RunBlue Dual Run & Blot apparatus (Expediton). The membrane was blocked with TBS containing 0.5% PVP10 at room temperature (RT) for 2 h. Sera were diluted 1:10 in TBST and pre-incubated with and without inhibitor extracts [C, WF, S and, as a control, bovine serum albumin (BSA) each at a concentration of 800 µg/mL] at 2–8°C, overnight. Serum-inhibitor mixes were then incubated with membranes at RT overnight with moderate shaking. After washing three times with TBST, membranes were incubated with AP-conjugated anti-dog IgE antibody (clone 5.91, 0.5 µg/mL in TBST) at RT for 2 h with shaking. Membranes were washed a final six times with TBST and rinsed twice with TBS prior to addition of NBT (nitro blue tetrazolium)/BCIP (5-bromo-4-chloro-3-indolyl-phosphate) substrate (Thermo Scientific; Rockford, IL, USA). The reaction was stopped with deionized water once bands of satisfactory intensity appeared (~1 to 2 min). Gel and immunoblots were analysed using Core Laboratory Image Quantification System (CLIQS, TotalLab Ltd; Newcastle, UK). Extract loading and inhibitor concentrations had been determined in earlier optimization experiments.

Allergen identification

Chicken, WF and S extracts were separated by SDS-PAGE and immunoblotting was performed using the two cross-reactive and control sera pools as described above.

Bands corresponding to IgE-binding proteins present in all three extracts (those of apparent 22, 34, 40, 50 and 65 kDa molecular weights) were excised from Coomassie blue-stained gels and sent to the Bioscience Technology Facility's Metabolomics and Proteomics Laboratory, Department of Biology, University of York, for mass spectrometry (MS) sequencing and protein identification. Briefly, this simply consisted of an in-gel digestion with trypsin to release the proteins and analysis using a short liquid chromatography (LC)-MS/MS acquisition.

The resulting tandem mass spectral data (i.e. linear amino-acid sequences) were matched to those in the Uniprot database (www.uniprot.org) using the Mascot programme (Matrix Science; London, UK) with either order-restricted (e.g. Aves) or unrestricted filtering. Only proteins whose threshold scores were above those of identity were examined further. Whenever the returned protein identification was not from the same species as that from the extract from which it was obtained, we performed a second manual search of both the Uniprot and the NCBI databases (www.uniprot.org, <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>; pages last accessed: April 24, 2018). In these searches, we attempted to match the identified sequences to those (if existing) from animals of the same species, genus or order. Following this approach, all samples except for one band (~65 kDa WF protein) yielded relevant protein identification. This band was then subsequently concentrated, purified and re-analysed using, this time, the matrix-assisted laser desorption/ionization (MALDI)-MS/MS instrument.

We then assessed if the newly identified proteins were known allergens for food-allergic humans by searching the following three allergen databases: www.allergen.org, www.allergome.org and www.allergenonline.org v1.88; these searches were last performed on April 24, 2018.

Once the proteins were identified by MS, available protein sequences were aligned between the three species (*Gadus morhua* being used for the WF) and the likelihoods of cross-reactivity between C, WF and S were determined based on the identity of their sequences and the results of immunoblotting inhibition (i.e. the

disappearance of the corresponding band after incubation with the extract of one or both nonhomologous species).

Statistical analyses

After verification of the lack of normal distribution of the IgE scores among 1,500 consecutive canine sera, Spearman's nonparametric correlations were determined, two at a time, between those with chicken, white fish and salmon in these sera. We used Prism 7 (GraphPad Software; La Jolla, CA, USA) with a degree of significance set at 5%.

Results

ELISA

We first wanted to verify if there was an association in the IgE sensitization patterns to C, WF and S allergens, as previous odds ratios of IgE scores had established a significant co-reactivity between these allergens.⁵

Most dogs did not exhibit any IgE reactivity to C, WF and S extracts, but a positive IgE score of ≥ 1 to these three allergens was found in 386 (26%), 303 (20%) and 328 (22%) of 1,500 dogs, respectively; the patterns of positive IgE scores were remarkably similar for the three allergens (Figure 1a). Comparing two-by-two the differences in categorical IgE scores, one can see that nearly 80% of the 1,500 sera had the same IgE score for the two allergens in question, the median difference in IgE scores for all three comparisons being 0 (Figure 1b–d).

Two-by-two correlations between pairs of allergens were highly significant, with Spearman coefficients of 0.68, 0.75 and 0.60 for C–WF, C–S and WF–S comparisons, respectively ($P < 0.0001$ for all three tests). Such a pattern suggests either a substantial cross-reactivity or co-sensitization among these sera for the three tested allergens.

In 53 of the canine sera shown to have at least one positive IgE score, most were polysensitized: 15 (28%) had elevated IgE to only one of the three allergens ("mono-positives"; Table 1), 12 (23%) against two allergens ("bi-positives") and 26 (49%) against all three allergens ("tri-positives"). White fish showed the highest frequencies of positive IgE reactivity (42 of 53; 79%), whereas S showed the lowest (37 of 53; 70%).

Inhibition ELISA

The pre-incubation of sera with WF and S inhibitors resulted in a $>50\%$ inhibition of IgE-binding to the C extract in seven of seven (100%) and four of seven dogs (57%), respectively (Figure 2). A greater than 50% inhibition of IgE-binding to WF was obtained in six of seven (86%) and five of seven dogs (71%) when the sera were pre-incubated with either C or S, respectively (Figure 3). With S as the immobilized coat allergen, a $>50\%$ inhibition was achieved in four of seven sera (57%) by pre-incubation with C and in six of seven sera (86%) by WF (Figure 4). The pre-incubation of sera with inhibitor extracts always led to a strong inhibition on homologous allergen coats (e.g. C inhibition of C; Table 2). In all seven tri-positive dogs, C and WF were the most effective inhibitors of serum IgE-binding to C coats (median inhibition of 83% and 71%, respectively; Table 2). The binding of IgE to the WF coat was most strongly inhibited by the

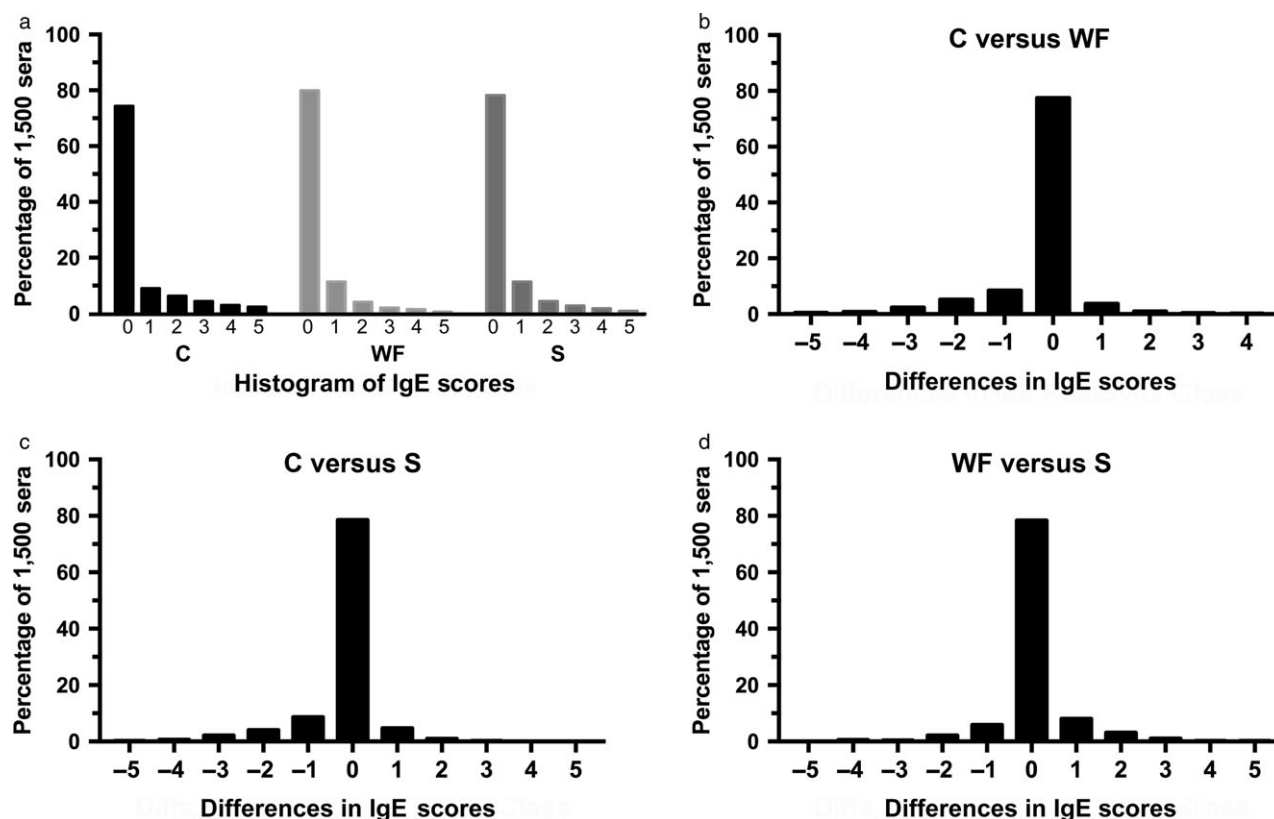


Figure 1. Determination of co-reactivity to chicken (C), white fish (WF) and salmon (S) extracts.

(a) Comparative histogram of IgE scores to the three allergens among 1,500 sera from allergy-suspected dogs; (b,c,d) differences in IgE scores in two-by-two comparisons: the median difference in IgE scores is "0", meaning that the IgE reactivity to both extracts is of the same magnitude in most sera.

Table 1. Chicken (C), white fish (WF) and salmon (S) IgE reactivity in 53 dogs with at least one positive IgE reaction, based on ELISA

	C	WF	S	C + WF	C + S	WF + S	C + WF + S
No. of positives	3	8	4	5	4	3	26
% positive	6%	15%	8%	9%	8%	6%	49%

n = 53 dogs. Positive >10.3 antibody units.

homologous WF (median: 97%) and S (median: 82%). The IgE-binding to S was most strongly inhibited by WF (median: 91%). In contrast, the negative control inhibitor allergen (i.e. potato) had minimal impact on the IgE-binding to either C, WF or S coats (medians 7%, 4% and 2% inhibition, respectively). Finally, the lack of IgE-binding to the extract coats by the negative control dog serum remained unaffected by pre-incubation with the various inhibitors.

SDS-PAGE

Extract proteins were separated according to molecular weight under reduced and denatured conditions. Multiple bands in the range of approximately 11–115 kDa were visible in the separated C extract, with distinct bands at approximately 22, 34, 40, 50, 65 and 72 kDa (Figure 5a: PAGE). With the WF extract (Figure 5b: PAGE), multiple bands between 7 and 115 kDa were observed, with individual bands corresponding to molecular weights of approximately 7, 13, 15, 17, 22, 34, 40, 50 and 65 kDa. Multiple bands between approximately 9 and 115 kDa were evident

where S proteins were separated, with clear bands of approximately 9, 22, 34, 40, 50, 65 and 95 kDa (Figure 5c: PAGE). All three extracts had five bands corresponding to proteins of approximately 22, 34, 40, 50 and 65 kDa. Both WF and S also had low molecular weight bands at approximately 7 kDa, a band not detected in the chicken extract.

Immunoblotting

The IgE-binding proteins in C and both fish extracts were analysed by immunoblotting using pooled sera from five dogs with detectable anti-C, anti-WF and anti-S IgE. Serum IgE reacted to nine C proteins between approximately 22 and 78 kDa (Figure 5a: Pos), eight WF proteins between 17 and 91 kDa (Figure 5b: Pos) and seven S proteins between 22 and 95 kDa (Figure 5c: Pos). IgE-binding proteins of approximately 18–22, 34, 40, 50 and 65 kDa were present in all food extracts. Based on the size of the bands, serum IgE reacted most strongly to three high molecular weight extract proteins of approximately 40, 50 and 65 kDa; IgE-binding to the 65 kDa S and

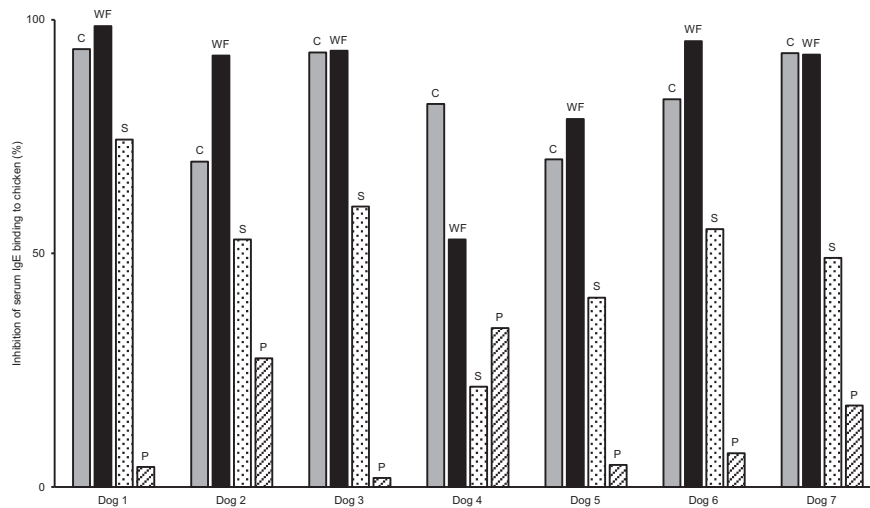


Figure 2. ELISA inhibition of IgE-binding to chicken (C) extract.

Percentage inhibition of dog serum IgE-binding to C (5 µg/mL) following overnight pre-incubation with homologous C inhibitor, white fish inhibitor (WF), salmon inhibitor (S) and the negative control inhibitor, potato (P). All inhibitors were used at a concentration of 1,000 µg/mL.

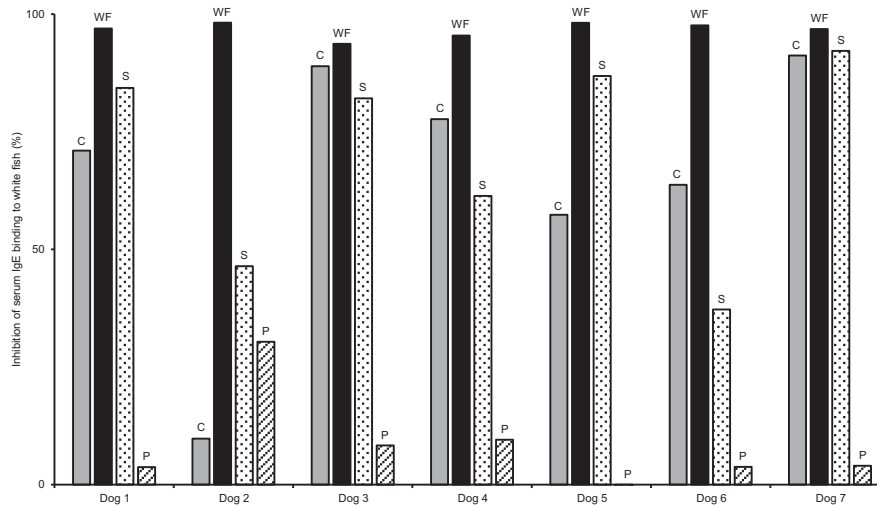


Figure 3. ELISA inhibition of IgE-binding to white fish (WF) extract.

Percentage inhibition of dog serum IgE-binding to WF (5 µg/mL) following overnight pre-incubation with chicken inhibitor (C), the homologous WF inhibitor, salmon inhibitor (S) and the negative control inhibitor, potato (P). All inhibitors were used at a concentration of 1,000 µg/mL.

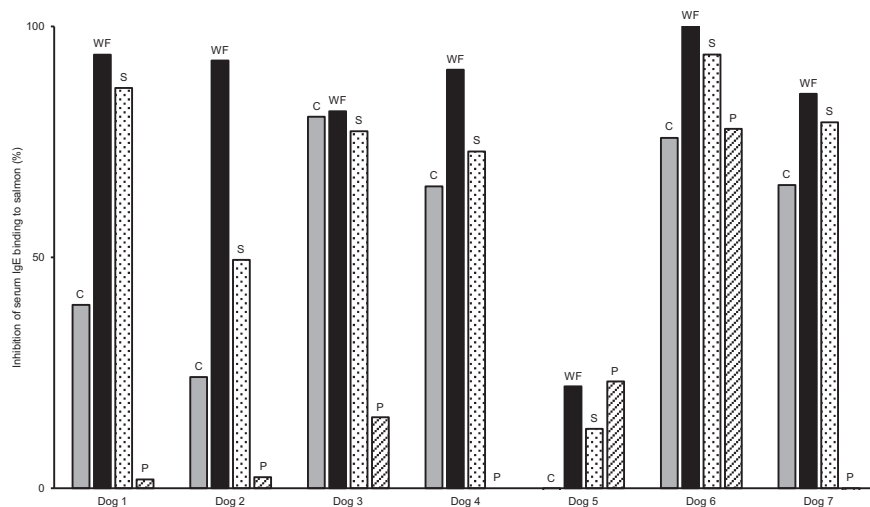


Figure 4. ELISA inhibition of IgE-binding to salmon (S) extract.

Percentage inhibition of dog serum IgE-binding to S (5 µg/mL) following overnight pre-incubation with chicken inhibitor (C), white fish inhibitor (WF), the homologous S inhibitor and the negative control inhibitor, potato (P). All inhibitors were used at a concentration of 1,000 µg/mL.

Table 2. Median percentage inhibition (range) of IgE-binding in chicken (C), white fish (WF) and salmon (S) co-positive dogs, based on ELISA inhibition

Inhibitor allergen	Coat allergen		
	C	WF	S
Potato	7 (2–34)	4 (0–30)	2 (0–78)
C	83 (70–94)	71 (10–91)	65 (0–81)
WF	93 (53–99)	97 (94–98)	91 (22–100)
S	53 (22–74)	82 (37–92)	77 (13–94)

n = 7 dogs. Allergens coated at 5 µg/mL. All inhibitors used at 1,000 µg/mL.

50 kDa C proteins was particularly intense. We did not detect any IgE reactivity to any of the C, WF and S proteins with the negative control serum pool (Figure 5a,b,c: Neg).

Inhibition immunoblotting

In order to identify cross-reactive IgE-binding proteins in the three extracts, inhibition immunoblots were performed with the same serum pool as that used for the previous immunoblotting experiment. Serum IgE-binding to C, WF and S extract proteins was almost completely abolished after the overnight pre-incubation with 800 µg/mL of their homologous inhibitor (Figure 6a: C; Figure 6b: WF; and Figure 6c: S). For the most reactive 40, 50 and 65 kDa C proteins, WF and S inhibitors resulted in a similar reduction of IgE-binding as that obtained with the homologous C inhibitor, the 40 and 50 kDa C proteins being only just visible (Figure 6a: WF and S inhibitors). Chicken strongly attenuated, but did not completely inhibit, IgE-binding to the 17, 40 and 50 kDa WF proteins (Figure 6b: C). Salmon inhibited all IgE reactivity to WF proteins (Figure 6b: S). White fish had a greater inhibitory effect on IgE-binding to the 40, 50 and 65 kDa S proteins (Figure 6c: WF) than C inhibitor (Figure 6c: C). Moreover, the inhibition of IgE-binding to S proteins by C (Figure 6c: C) was less effective than the reciprocal inhibition of IgE-binding to C proteins by S (Figure 6a: S). Finally, the pre-incubation of the sera with the BSA control inhibitor had no visible effect on IgE-binding to either C, WF or S proteins (Figure 6a, 6b and 6c, respectively: BSA) when compared with the corresponding uninhibited control (Figure 6a, 6b and 6c, respectively).

Allergen identification

Mass spectrometry results permitted the specific identification of nine different proteins with at least two matched peptides equal or greater than the threshold values for a 95% identity confidence threshold for at least one of the species of interest (Table 3).

For the proteins in the C extract, the specific identification of a *Gallus domesticus* protein was achieved for seven proteins; for S, a match to a *Salmo salar* protein was obtained in five cases.

For WF, there were no specific matches for the proteins of the two species (*Gadus morhua* and *Melanogrammus aeglefinus*) present in the extract, but this was due to the absence of the corresponding protein sequences in the Uniprot database. In spite of this caveat, the peptide sequences matched to those of either C (two

proteins), *Danio rerio* zebrafish (two proteins) or to those of multiple fish species (two proteins). For two bands, the identified proteins were matched to a nonchicken and nonfish species peptide; these were not investigated further.

We then looked for the newly identified proteins in the three main allergen databases. Two proteins were known allergens in the three species of interest: the (beta) enolase 3 (ENO3: Gal d 9 and group 2 fish allergens Gad m 2 and Sal s 2 in C, cod and S, respectively) and the fructose-bisphosphate aldolase A (ALDOA: Gal d 10 and group 3 fish allergen Gad m 3 and Sal s 3). Two other proteins were recognized C (Gal d GAPDH) or cod (Gad m CK) allergens. Even though all other proteins have not yet been identified as C, cod or S allergens in food-allergic human patients, homologous proteins had been recognized previously as allergens in one or more other fish, shellfish, plant, mould, mammalian, insect and even house dust mite species (data not shown).

Altogether, taking into account both inhibition immunoblotting results and the percentage of amino-acid sequence identity between these proteins, the likelihood of partial or full cross-reactivity between these nine allergens was high; this is depicted as a heat map (Figure 7a and b).

Discussion

We first began verifying that, among a large number of sera collected from dogs suspected of allergy, there was a common co-reactivity to C, WF and S, an association that had been reported previously.⁵ Indeed, IgE sensitization patterns to these three allergens were usually either nonreactive or reactive to more than one of these allergens. The correlations between IgE score pairs were highly significant, thereby confirming the previously known association of IgE sensitization existing between these allergens in dogs⁵ and humans.¹³

In subsequent inhibition ELISA experiments, we established that seven polyreactive sera contained IgE that recognized, albeit not exclusively, cross-reactive allergens. Such cross-reactivity was confirmed in immunoblotting inhibition by the disappearance of specific bands with heterologous, but not control, inhibitors. Mass spectrometry then permitted the sequencing of protein(s) corresponding to the bands that had disappeared after pre-incubation with the inhibitors.

Altogether and with this combination of experiments, we established the presence of at least nine cross-reactive canine serum IgE-binding allergens present in extracts of C, WF and S.

Most of these proteins are known to be involved in the latter stages of glycolysis in muscles. The 65 kDa protein, pyruvate kinase, catalyzes the direct transfer of phosphate from phosphoenolpyruvate to ADP to produce ATP and pyruvate. It has previously been identified as a human serum IgE-reactive protein in prawn²⁰ and acknowledged as a novel allergenic protein in shrimp.²¹

Human studies also have identified the 50 kDa beta-enolase (enolase 3, ENO3) as a major allergen in C (Gal d 9), Atlantic cod (Gad m 2) and S (Sal s 2).²² Another glycolytic enzyme, enolase exists in three

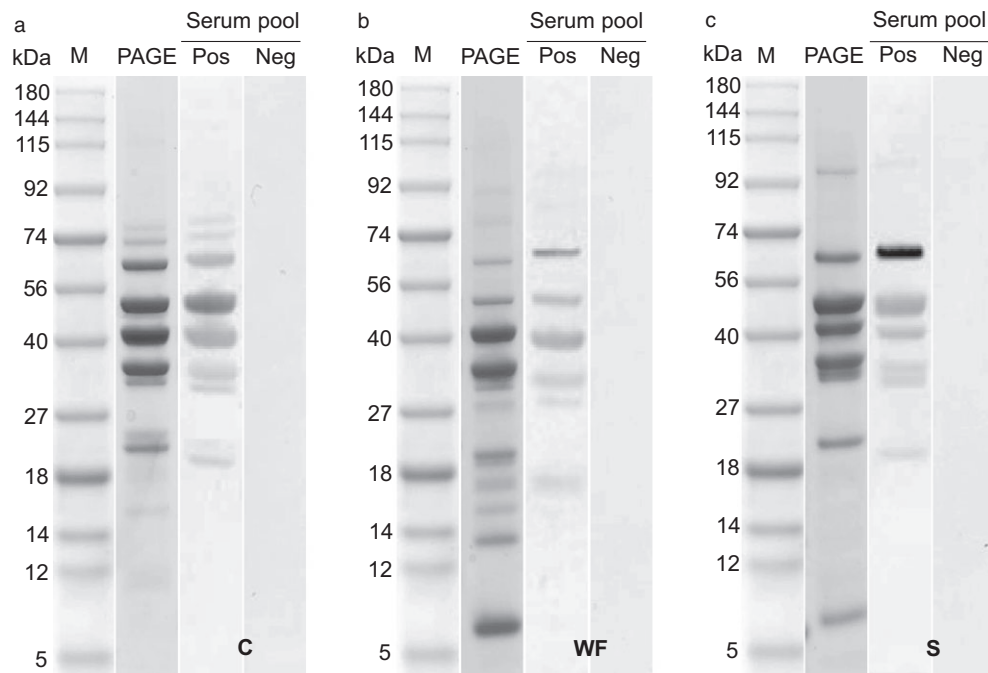


Figure 5. Immunoblotting with chicken (C), white fish (WF) and salmon (S) extracts.

Separation of C (a), WF (b) and S (c) extract proteins (2 µg/lane) in 4%–12% gels by SDS–PAGE (lanes PAGE). Proteins were transferred to PVDF membranes and incubated with a serum pool previously shown to have IgE co-reactivity to C, WF and S (Pos) and a pool of dog sera previously shown to have no IgE reactivity to the extracts (Neg). Serum IgE-binding bands in each extract were detected using alkaline-phosphatase conjugated anti-dog IgE (clone 5.91; 0.5 µg/mL) and NBT/BCIP substrate. Molecular weight markers are shown in lanes M.

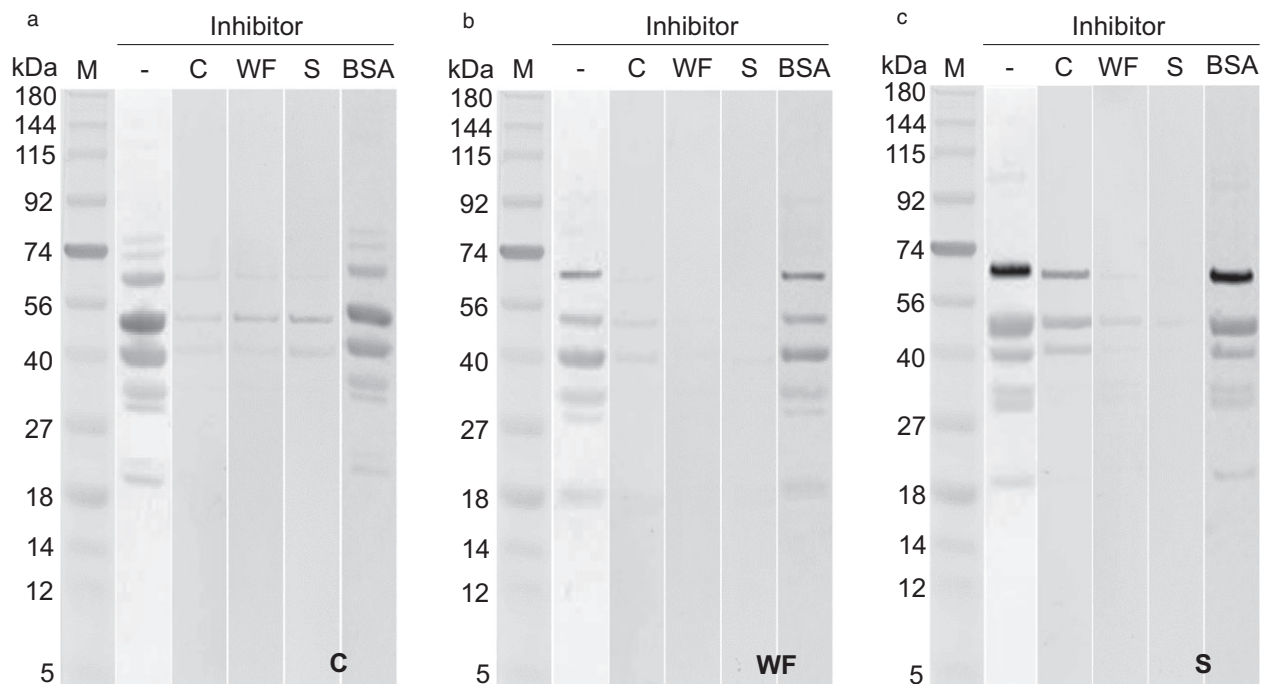


Figure 6. Immunoblotting inhibition with chicken (C), white fish (WF) and salmon (S) extracts.

Inhibition of IgE-binding to proteins in C (a), WF (b) and S (c) extracts following overnight pre-incubation of a positive dog serum pool without inhibitor (i.e. in buffer alone; lanes -), with C inhibitor, with WF inhibitor and with S inhibitor extracts. Bovine serum albumen was used as a negative control inhibitor. All inhibitors were used at a concentration of 800 µg/mL.

isoforms: alpha-enolase, a ubiquitous isoform present in all tissues; muscle-specific beta-enolase; and gamma-enolase, present in nervous and neuroendocrine tissue.

The C and S proteins at approximately 40 kDa correspond to the enzymes creatine kinase M-type (CKM) or fructose-bisphosphate aldolase A (ALDOA). Aldolase has previously been identified as an IgE-binding allergen by

Table 3. Allergen identification

Theoretical MW (kDa)	Immunoblotting MW (kDa)	Protein(s)	Chicken extract	White fish extract	Salmon extract
58	65	Pyruvate kinase M 1/2	Protein sequence match with that of <i>Gallus domesticus</i> (chicken)	<i>Gadus morhua</i> protein sequence not available in database; query identified protein sequences for <i>Gallus domesticus</i>	No match to <i>Gallus domesticus</i> or fish species sequences
47	50	Enolase 3	Protein sequence match with that of <i>Gallus domesticus</i>	<i>Gadus morhua</i> protein sequence not available in database	Protein sequence match with that of <i>Salmo salar</i> (salmon)
43	40	Creatine kinase, M type	Protein sequence match with that of <i>Gallus domesticus</i>	<i>Gadus morhua</i> sequence not available in database	Protein sequence match with that of <i>Gallus domesticus</i>
40	40	Aldolase, fructose-bisphosphate A	<i>Gallus domesticus</i> protein sequence not available in database	<i>Gadus morhua</i> protein sequence not available in database	Protein sequence match with that of <i>Salmo salar</i>
42	40	Actin, alpha 1, skeletal muscle	Protein sequence match with that of <i>Gallus domesticus</i>	<i>Gadus morhua</i> protein sequence not available in database; query identified protein sequences for <i>Gallus domesticus</i> and several fish species	<i>Salmo salar</i> protein sequence not available in database
36	34	Glyceraldehyde-3-phosphate dehydrogenase	Protein sequence match with that of <i>Gallus domesticus</i>	<i>Danio rerio</i> (Zebra fish)	Query identified protein sequences for several fish species
36	34	Malate dehydrogenase 2	No match to protein sequence of <i>Gallus domesticus</i> or any fish species	<i>Gadus morhua</i> protein sequence not available in database	Query identified protein sequence for <i>Danio rerio</i> (Zebra fish)
36	34	Lactate dehydrogenase A	<i>Gallus domesticus</i>	<i>Gadus morhua</i> protein sequence not available in database; query identified protein sequence for several fish species	Protein sequence match with that of <i>Salmo salar</i>
27	22	Triose phosphate isomerase 1	<i>Gallus domesticus</i>	Sequence not available in database; query identified protein sequence for <i>Danio rerio</i>	Protein sequence match with that of <i>Salmo salar</i>

Allergens were initially identified by SDS–PAGE and immunoblotting with sera reacting to chicken, fish and salmon extracts; then bands corresponding to IgE-binding proteins present in all three extracts (those of apparent 22, 34, 40, 50 and 65 kDa molecular weights) were excised and analysed by mass spectrometry and sequenced.

Not available: a sequence greater than 10% of the protein sequence of the species present in the extract is not available in the Uniprot database. MW molecular weight.

2D-PAGE in a Pacific salmon species,²³ whereas the CKM is a recognized allergen in tuna.²⁴ Aldolase is another glycolytic enzyme and, in humans, is a known cross-reactive allergen between C (Gal d 10) and fish (cod: Gad m 3; S: Sal s 3).¹³

The IgE-binding protein(s) in WF, which migrate(s) at approximately 40 kDa are either CKM or the muscle protein actin, which is a novel cod allergen for dogs. Given that cod actin has a 99% sequence homology with the corresponding S and C protein, it is likely to be highly cross-reactive. Clam,²⁵ crab,²⁶ beef²⁷ and Nile perch²⁸ actins are recognized allergens for humans. Unlike for C and S extracts, aldolase was not found to be at this molecular weight in the WF extract, but this was likely due to the absence of the sequence in the protein databases used for matching MS-derived amino-acid sequences. Inhibition immunoblots showed that the WF extract induced a total inhibition of IgE-binding to the chicken and salmon 40 kDa proteins, thereby indicating the presence of (a) shared protein(s). As CKM was identified as a potential match in all three extracts, it could be

assumed that this would be the most likely protein, but this could also have been due to the fact that aldolase was not included in the databases for all three extracts.

The 34 kDa C protein was matched to the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which previously has been reported not only as a novel fish allergen,²⁹ but also one in shrimp, some plants (e.g. latex, wheat, cassava), insects, moulds and several mammalian meats (www.allergome.com).

Finally, triose phosphate isomerase (17–22 kDa) was identified in all three extracts and is a recognized allergen in fish,²³ midges,³⁰ crustaceans,³¹ various plants,^{32,33} and as a novel allergen in octopus.³⁴

We were surprised to find highly-conserved proteins such as GAPDH (with a 93, 76 and 62% identity between its amino-acid sequence in C and beef, wheat and the mould *Alternaria alternata*, respectively) to be IgE pan-allergens. Another notable example, among many others, is the 71% sequence identity existing between the triose phosphate isomerase 1 of C and that of *Dermatophagoides farinae* house dust mite allergen Der f 25. Altogether, such

unfortunate and very common high rates of sequence identity between these conserved pan-allergens raises the spectre of many unpredictable cross-reactive allergies in patients with IgE sensitization to a pan-allergen in one food item, and potential clinical reactivity to this and many other food or environmental allergen sources.

Most if not all of the strongest IgE-binding proteins in these extracts are enzymes. The dual enzymatic and allergenic properties of some proteins have been known for many years and mechanisms whereby the enzymatic activity could increase allergenic potency have been described. Perhaps the best-known example is the house dust mite allergen, Der p 1, a proteolytic enzyme capable of cleaving CD23 (FcεRII, the low-affinity IgE receptor) and CD25 (IL-2 receptor α-chain) expressed on the surface of some B cells,³⁵ resulting in dysregulation of the immune response and induced production of IgE.³⁶ The allergenicity of these proteins is not limited to their enzymatic activity, however, and both relative abundance and stability have been cited as of equal importance.^{37,38} Pyruvate kinases, enolases and aldolases have been shown to be abundant but heat-labile proteins in fish²² and shrimp.²¹

The objective of this work was to identify whether cross-reactive IgE-binding proteins were present in C and

fish, which could explain the frequency of sensitizations to these foods. The inhibition ELISA data were consistent with the presence of cross-reactive IgE-binding epitopes in C, WF and S. With the exception of the negative control inhibitor, S was the least effective at inhibition of IgE-binding to heterologous coats. Moreover, WF was more effective at inhibiting IgE-binding to S than the homologous S inhibitor, suggesting that serum IgE had a stronger binding affinity to WF proteins and/or a greater abundance of cross-reactive proteins in WF. The higher frequency of serum IgE reactivity to WF, in addition to its efficacy as an inhibitor of heterologous proteins, could reflect its current prevalence in the diets of the dogs used in this study.

For inhibition ELISAs, potato was chosen as a negative control food allergen extract inhibitor. This choice was, in part, guided by the fact it is a nonmeat food extract and that previous ELISA analysis of the individual sera from the pool showed levels of potato-specific IgE to be below the positive threshold of the assays. Accordingly, serum IgE-binding to C, WF and S coats was largely unaffected by the presence of potato extract inhibitor. When used for immunoblot inhibitions, however, potato was shown to have a minor inhibitory effect on IgE-binding to many

Protein(s)	a) IgE Immunoblotting Cross-Reactivity			b) Sequence identity		
	Chicken X White Fish	Chicken X Salmon	Salmon X White Fish	Chicken X White Fish	Chicken X Salmon	Salmon X White Fish
Pyruvate kinase M 1/2				NA	82%	NA
Enolase 3				NA	81%	NA
Creatine kinase, M type				83% (Gm)	84%	91% (Gm)
Aldolase, fructose-bisphosphate A				NA	NA	NA
Actin, alpha 1, skeletal muscle				98% (Gm)	99%	99% (Gm)
Glyceraldehyde-3-phosphate dehydrogenase				86% (Gm)	86%	91% (Gm)
Malate dehydrogenase 2				NA	80%	NA
Lactate dehydrogenase A				75% (Pm)	74%	87% (Pm)
Triose phosphate isomerase 1				NA	82%	NA
	fully IgE cross-reactive			76-100% identity		
	partially IgE cross-reactive			51-75% sequence identity		
	non IgE cross-reactive			0-50% sequence identity		

Figure 7. Heat map of cross-reactive IgE-binding between chicken (C), white fish (WF) and salmon (S) proteins (a) and the likelihood of cross-reactivity based on sequence identity (b).

NA full sequence not available at genus or species level, Gm *Gadus morhua*, Pm *Paranotothenia magellanica* (Maori cod also known as *Gadus magellanicus*).

of the extract proteins (data not shown). It was not clear if this was due to a particularly strong blocking effect or the unintentional identification of potential serological cross-reactivity with potato proteins. For this reason, inhibition blots were repeated with BSA as a negative control inhibitor, which resulted in little inhibition of band signal. Mass spectrometry revealed that most IgE-reactive proteins in C and fish are enzymes involved in the metabolism of glucose, thus raising the possibility that they could be sources of sensitization in other species of poultry and fish, and, indeed, any organism that metabolizes glucose into energy. Glycolytic enzymes including enolases, triose phosphate isomerase and fructose 1, 6-bisphosphate have been found in the tubers of root crops including potatoes³⁹ where large amounts of energy are required during starch accumulation,⁴⁰ but they are not thought to be major allergens in potato. The relatively high inhibition of IgE-binding by C and fish proteins compared with potato does, however, suggests that such cross-reactivity, if any, would be very low. Nevertheless, this observation further illustrates the complexity of immunological cross-reactivity between foods.

It was notable that all canine serum IgE-binding observed in this study was directed to extract proteins of molecular weight >20 kDa. This is in contrast to studies in humans where the major allergenic component in fish is the ~12 kDa protein, parvalbumin.²² By SDS-PAGE, the WF extract, in particular, was shown to contain a number of low molecular weight proteins, although none were recognized by canine serum IgE. Mass spectrometry analysis of the 13 kDa WF band was undertaken to ascertain its identity, but it was not matched to parvalbumin. For humans, the allergenicity of fish is thought to be related to its parvalbumin content,⁴¹ with highest levels being found in dorsal and rostral white muscle, and comparatively little in dark muscle.⁴² Its detection could therefore be dependent upon the amount of parvalbumin-rich muscle present in the allergenic extract. Levels of parvalbumin in S have been found to be very low with respect to both content and IgE reactivity,⁴³ which could account for the lack of a 12 kDa band in the S extract. Similarly, C parvalbumin has been found to be detectable only in leg and wing meats, but not in C breast.¹³ Again, it is possible that the commercial whole C extract used here contained a high quantity of breast muscle tissue and little leg or wing material. The apparent lack of IgE reactivity to low molecular weight proteins that correspond to a major food constituent of human fish allergy could therefore be due to under-representation of component protein in the extracts rather than a lack of specific IgE reactivity. Assays using purified parvalbumin would be needed to confirm whether this protein is also an allergen for dogs. The use of whole-allergen extracts, in which concentrations of specific and stable allergenic proteins are unknown, could play a part in the poor diagnostic accuracy of *in vitro* allergen-specific IgE tests. There is increasing evidence that IgE sensitization to some food proteins might be more important than others with respect to clinical manifestations of allergy. In humans, for example, measurement of component-specific IgE responses to ovomucoid (Gal d 1) and/or ovalbumin (Gal d 2) have been shown to improve the diagnostic accuracy in patients with a history of severe and/or immediate reactions to

hen's egg.⁴⁴ Similarly, measurement of IgE reactivity to Ara h 2 was more predictive of peanut allergy than either skin prick testing and specific IgE to whole peanut extract.⁴⁵ The assessment of IgE reactivity to allergen components of food (the so-called "component-resolved diagnostics") rather than to whole allergen extracts, whose allergen content could be variable, is likely to permit the differentiation between IgE reactivity to food components that initiate systemic allergic responses from those with little allergenic potential and, ultimately, a more accurate diagnosis of food allergy.^{46,47}

In conclusion, this study provides evidence of serological IgE reactivity of dogs to homologous proteins in C, WF and S. As sera without a firm diagnosis of food allergy were used, however, the clinical relevance of allergenicity of these cross-reactive IgE-binding proteins remains to be determined in dogs with demonstrable signs of allergy upon ingestion of pairs of these food sources.

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Résumé

Contexte – Chez l'homme, une allergie clinique réactive croisée a été décrite entre trois protéines de poulet et de poisson : beta-énolase, aldolase A et parvalbumine.

Objectif – Evaluer si la réactivité croisée IgE entre le poulet et le poisson existe aussi chez le chien.

Sujets – Les sera de chiens suspects de dermatose allergique avec des IgE anti-poulet et poisson.

Matériel et méthode – Les sera ont été analysés par ELISA et immunoblotting avec le poulet, le poisson

blanc (haddock et morue) et le saumon. Les ELISA inhibiteur réciproque et l'immunoblotting inhibiteur ont été réalisés. Le séquençage des protéines et l'identification des bandes sur extraits multiples a été déterminé par spectrométrie de masse.

Résultats – Sur les 53 sera canins archivés testés par ELISA contre le poulet, le poisson blanc et le saumon, respectivement, 15 (28%), 12 (23%) et 26 (49%), avaient des IgE élevés contre un, deux ou les trois extraits. Sept des sera triple-réactifs ont été soumis à l'ELISA d'inhibition réciproque. Une inhibition >50% a été trouvée entre le poulet-poisson, poulet-saumon et poisson blanc-saumon respectivement pour les sept, quatre et cinq des sept chiens. L'immunoblotting a identifié les protéines porteuses d'IgE multiple comme des protéines de poids moléculaire identiques dans trois extraits ; ceux-ci ont été partiellement à totalement cross-réactifs par immunoblotting inhibiteur. La spectrométrie de masse a identifié neuf protéines cross-réactives : pyruvate kinase, créatine kinase, alpha-actine, glyceraldehyde-3-phosphate dehydrogénase, beta-énolase, aldolase, malate dehydrogénase, lactate dehydrogénase et triose-phosphate isomérase 1. Tous ces éléments ont été rapportés précédemment chez l'homme pour les allergènes de poisson, les crustacés et /ou le poulet.

Conclusion et importance clinique – Si un de ces nouveaux allergènes cross-réactifs poulet-poisson est la cause d'allergie clinique doit être déterminée chez les chiens réagissant à au moins deux de ces sources fréquentes de nourriture.

Resumen

Introducción – en humanos, se ha descrito una alergia clínica por reacción cruzada entre tres proteínas de pollo y carne de pescado: beta-enolasa, aldolasa A y parvalbúmina.

Objetivo – evaluar si existe reactividad cruzada de IgE entre el pollo y el pescado en perros.

Animales – Sueros de perros con sospecha de enfermedad alérgica de la piel y con IgE contra pollo y pescado.

Métodos y materiales – Los sueros se analizaron mediante ELISA e inmunotransferencia con extractos de pollo, pescado blanco (eglefino y bacalao) y de salmón. Posteriormente se realizaron ELISA de inhibición recíproca e inmunoblots de inhibición. La secuenciación de proteínas de las bandas identificadas en extractos múltiples se determinó mediante espectrometría de masas.

Resultados – De los 53 sueros caninos archivados probados por ELISA contra pollo, pescado blanco o salmón, 15 (28%), 12 (23%) y 26 (49%), respectivamente, tenían IgE elevada contra uno, dos o los tres de estos extractos. Siete de los sueros triplemente reactivos se sometieron a ELISA de inhibición recíproca. Se encontró una inhibición > 50% entre pollo y pescado, salmón y pollo y salmón y pescado en siete, cuatro y cinco de siete perros, respectivamente. La inmunotransferencia identificó proteínas de unión a IgE múltiples de pesos moleculares idénticos en los tres extractos; estos fueron parcialmente a completamente reactivos cruzados por inmunoblotting de inhibición. La espectrometría de masas identificó nueve proteínas de reacción cruzada como: piruvato quinasa, creatina quinasa, alfa-actina, gliceraldehído-3-fosfato deshidrogenasa, beta-enolasa, aldolasa, malato deshidrogenasa, lactato deshidrogenasa y triosa fosfato isomerasa 1. Todos estos han sido reportados previamente como alérgenos de pescado, marisco y/o pollo para humanos.

Conclusiones e importancia clínica – para establecer si alguno de los alérgenos con reacción cruzada de IgE identificados en este estudio son causa de alergia clínica, sería necesario determinar si los perros reaccionan al menos a dos de estas fuentes de alimentos comunes.

Zusammenfassung

Hintergrund – Beim Menschen wurde eine kreuzreaktive klinische Allergie zwischen drei Huhn- und Fischfleischproteinen beschrieben: Beta-Enolase, Aldolase A und Parvalbumin.

Ziel – Eine Evaluierung einer möglicherweise existierenden IgE Kreuzreaktion zwischen Huhn und Fisch beim Hund.

Tiere – Sera von Hunden mit Verdacht auf eine allergische Hauterkrankung und mit IgE gegen Huhn und Fisch.

Methoden und Material – Die Sera wurden mittels ELISA und Immunoblot mit Huhn, weißem Fisch (Schellfisch und Kabeljau) und Lachsextrakten analysiert. Danach wurden reziproke Inhibitions ELISAs und Inhibitionsimmunoblots durchgeführt. Sequenzierung von Proteinen der Bande, die in multiplen Extrakten identifiziert wurden, wurden mittels Massenspektrometrie bestimmt.

Ergebnisse – Von den 53 archivierten Hundesera, die mittels ELISA auf Huhn, weißen Fisch bzw Lachs getestet worden waren, zeigten 15 (28%), 12 (23%) bzw 26 (49%) erhöhte IgE Werte gegenüber einem, zwei oder allen drei dieser Extrakte. Sieben der dreifach-reaktiven Sera wurden mittels reziprokalem Inhibitions ELISA untersucht. Eine > als 50%ige Inhibition wurde zwischen Huhn-Fisch, Huhn-Lachs bzw Fisch-Lachs bei sieben, vier bzw fünf der sieben Hunde gefunden. Mittels Immunoblot wurden multiple IgE-bindende Proteine von identischem molekularem Gewicht in den drei Extrakten gefunden; diese waren teilweise bis völlig kreuzreaktiv mittels Inhibitionsblot. Die Massenspektrometrie identifizierte neun kreuzreaktive Proteine: Pyruvatkinase, Creatinkinase, Alpha-Aktin, Glyceraldehyd-3-Phosphat

Dehydrogenase, Beta-Enolase, Aldolase, Malatdehydrogenase, Laktatdehydrogenase und Triose-Phosphat Isomerase 1. All diese sind bereits zu einem früheren Zeitpunkt als Fisch, Krustentier und/oder Huhn Allergene für Menschen beschrieben worden.

Schlussfolgerungen und klinische Bedeutung – Ob einige dieser neu identifizierten IgE kreuzreaktiven Huhn-Fisch Allergene ursächlich sind für eine klinische Allergie muss bei Hunden, die auf mindestens zwei dieser häufigen Futterquellen reagieren, noch bestätigt werden.

要約

背景 – 人医療領域において、3つの鶏および魚タンパク質(b-エノラーゼ、アルドラーゼAおよびパルプアルブミン)間において、交差反応性アレルギーによる臨床症状が報告されている。

目的 – 本研究の目的は、鶏および魚間のIgE交差反応性が犬においても存在するか評価することである。

被験動物 – アレルギー性皮膚疾患と鶏および魚に対するIgEの保持を疑わせる犬由来血清。

方法および材料 – 血清は鶏、白身魚(ハドックおよびタラ)およびサーモン抽出物に対するELISA法およびimmunoblot法によって解析した。その後Reciprocal inhibition ELISA法およびinhibition immunoblot法を実施した。複数の抽出物から同定したバンドのタンパク質配列決定は質量分析法によって決定した。

結果 – 鶏、白身魚またはサーモンに対してELISA法を実施した犬血清53頭中、15頭(28%)、12頭(23%)および26頭(49%)が1、2または3つ全ての抽出物に対してそれぞれIgEが上昇していた。3つ全ての抽出物に反応した血清のうち7つをReciprocal inhibition ELISA法に供した。7頭の犬のうち7、4、および5頭において、鶏 - 魚、鶏 - サーモンおよび魚 - サーモン間でそれぞれ> 50%の阻害が見出された。3つの抽出物における同一分子量の複数のIgE結合タンパクを、immunoblot法により同定した;これらはinhibition immunoblot法によって部分的もしくは完全な交差反応性を認めた。質量分析法により、ビルビン酸キナーゼ、クレアチンキナーゼ、a-アクチン、グリセルアルデヒド-3-リン酸デヒドロゲナーゼ、b-エノラーゼ、アルドラーゼ、リンゴ酸デヒドロゲナーゼ、乳酸デヒドロゲナーゼおよびトリオース - リン酸イソメラーゼ1を同定した。これらは全て、人の魚類、甲殻類および/または鶏のアレルゲンとしてこれまでに報告されている。

結論と臨床的重要性 – 新たに同定したIgE交差反応性鶏-魚アレルゲンのいずれかがアレルギーによる臨床症状をもたらすかどうかは、共通した食物源の少なくとも2つに反応を示す犬において決定する必要がある。

摘要

背景 – 有报道称,在三种鸡肉和鱼肉蛋白:β-烯醇化酶、醛缩酶A和小清蛋白之间,人类存在交叉反应性的临床过敏。

目的 – 评估犬的鸡肉和鱼肉IgE之间是否存在交叉反应。

动物 – 选择怀疑患有过敏性皮肤病的犬,抽取其血清检测鸡肉和鱼肉IgE。

材料和方法 – 分别通过ELISA和免疫印迹法检测血清的鸡肉、白鱼(黑线鳕和鳕鱼)和鲑鱼提取物,进行分析;然后进行交互抑制ELISA和抑制免疫印迹法。通过质谱法测定多种提取物的蛋白序列带。

结果 – 共有53份存档的犬血清,ELISA检测其鸡、白鱼或鲑鱼提取物,其中15份(28%)犬血清有一种提取物IgE升高;12份(23%)犬血清有两种提取物IgE升高;26份(49%)犬血清有三种提取物IgE升高。三反应性血清中有7份进行交互抑制ELISA试验,7份犬血清中的7、4和5份分别在鸡-白鱼、鸡-鲑鱼和白鱼-鲑鱼中发现> 50%的抑制作用。免疫印迹法鉴定了三种提取物中具有相同分子量的多种IgE结合蛋白;这些通过抑制免疫印迹法部分达到完全交叉反应。质谱法鉴定出九种交叉反应蛋白:丙酮酸激酶、肌酸激酶、a-肌动蛋白、甘油醛-3-磷酸脱氢酶、b-烯醇酶、醛缩酶、苹果酸脱氢酶、乳酸脱氢酶和丙糖磷酸异构酶1,含有这些成分鱼类、贝类和/或鸡肉都被报道为人类过敏原。

结论和临床价值 – 这些新发现的鸡-鱼过敏原IgE交叉反应,是否是导致临床过敏的原因,需要在犬对至少两种常见食物存在反应时确定。

Resumo

Contexto – Em humanos, há relatos de manifestações clínicas de alergia relacionados a reações cruzadas entre três proteínas de carne de frango e peixe: beta-enolase, adolase A e parvalbumina.

Objetivo – Avaliar se também existe reação cruzada de IgE entre frango e peixe em cães.

Animais – Soro de cães com suspeita de dermatopatia alérgica e apresentando IgE contra peixe e frango.

Métodos e materiais – O soro foi analisado por ELISA e imunoblotting com extratos de frango, peixe branco (haddock e bacalhau) e salmão. ELISA de inibição recíproca e imunoblots de inibição foram realizados. O sequenciamento proteico das bandas identificadas nos extratos múltiplos foi determinado por espectrometria de massa.

Resultados – Dos 53 soros caninos testados por ELISA contra frango, peixe branco e salmão, 15 (28%), 12 (23%) e 26 (49%), respectivamente, apresentaram IgE contra uma, duas ou três destes extratos. Sete dos soros triplamente reativos foram submetidos a ELISA de inibição recíproca. Inibição > 50% foi observada entre frango-peixe, frango-salmão e peixe-salmão em sete, quatro e cinco dos sete cães, respectivamente. O imunoblotting identificou múltiplas proteínas ligadas IgE de pesos moleculares idênticos em três extratos; estes apresentaram reações cruzadas parciais a completas no imunoblotting de inibição. A espectrometria de massa identificou nove proteínas com reação-cruzadas: piruvato quinase, creatina

quinase, alfa-actina, gliceraldeído-3-fosfato desidrogenase, beta-enolase, aldolase, malato desidrogenase, lactato desidrogenase e triose-fosfato isomerase 1. Todas estas já foram previamente relatadas como alérgenos de peixe, crustáceos e/ou frango para humanos.

Conclusões e importância clínica – É necessário se investigar se algum destes alérgenos de peixe-frango com reação cruzada é o fator causal de manifestações clínicas de alergia em cães reativos a pelo menos dois destes alimentos comuns.