

Co-sensitization and cross-reactivity between related and unrelated food allergens in dogs – a serological study

Jennifer Bexley*, Timothy J. Nuttall†, Bruce Hammerberg‡ and Richard E. Halliwell†

*Avacta Animal Health, Unit 706, Avenue E West, Thorp Arch Estate, Wetherby, LS23 7GA, UK.

†Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Edinburgh, EH25 9RG, UK.

‡College of Veterinary Medicine, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607, USA.

Correspondence: Jennifer Bexley, Avacta Animal Health, Unit 706, Avenue E West, Thorp Arch Estate, Wetherby, LS23 7GA, UK. E-mail: jennifer.bexley@avacta.com

Background – Knowledge of cross-reactivity between foods is useful so that potentially cross-reactive allergens can be avoided in diet trials.

Hypothesis/Objectives – To evaluate allergenic cross-reactivity in related foods.

Animals – Sera from 469 dogs with suspected adverse food reactions.

Methods – An IgE-based serological assay using 19 food allergens was performed in 469 dogs. Pairwise comparisons were used to calculate the odds ratios (ORs) for each food pair, with significance at $P < 0.0002$ by Holm–Bonferroni correction, both in all 469 dogs and in the 261 of 469 dogs with at least one positive reaction. One-way ANOVA with Tukey's post hoc tests (significance at $P < 0.05$) were used to test for differences between mean logE ORs in different food groups. Inhibition enzyme-linked immunosorbent assays (ELISAs) were performed to assess allergenic cross-reactivity between beef, lamb and cow's milk.

Results – Significant associations were observed between both related and unrelated food pairs. Associations were, however, more frequent and stronger among related than unrelated foods. In all 469 dogs, 38 of 43 related food pairs were significantly associated [mean (SD) logE OR 3.4 (0.9)] compared with 79 of 128 unrelated pairs [2.7 (1.0)], $P < 0.0002$. In positive dogs, 32 of 43 related pairs were significantly associated [2.7 (1.0)] compared with 49 of 128 unrelated pairs [1.8 (1.0)], $P < 0.0002$. Inhibition ELISAs confirmed the presence of cross-reactive IgE-binding epitopes in beef, lamb and cow's milk.

Conclusions and clinical importance – The results suggest that related and potentially cross-reactive foods should be avoided in elimination diets.

Introduction

Cutaneous adverse food reactions (AFRs) are a common cause of nonseasonal pruritus in dogs and can result from both immunological and nonimmunological reactions. In a recent study of 259 dogs with allergic dermatitis where flea bite hypersensitivity was excluded, 70.7% were diagnosed with aeroallergen-induced canine atopic dermatitis (CAD), 25.1% with an AFR and 4.2% with both conditions.¹ The clinical signs of CAD and AFRs in dogs can be indistinguishable, making it challenging to achieve a precise diagnosis.

The immunological mechanisms involved in cutaneous AFRs are complex and poorly understood. Early studies

did not support a role for IgE in the pathogenesis,^{2,3} but the diagnosis of AFR in these dogs was based upon a 3 week elimination diet trial as opposed to the eight to 10 weeks now considered mandatory.⁴ Another study demonstrated good correlation between dietary provocation and levels of allergen-specific IgE and immunoblots.⁵ Additionally, results of lymphocyte blastogenesis and patch testing in dogs with AFR have suggested a role for cell-mediated hypersensitivity.^{6–8} Cell-mediated reactivity is likely to be accompanied to some extent by antibody production and the negative predictive value of allergen-specific IgE and IgG is reportedly 80.7% and 83.7%, respectively.⁸

Despite these immunological correlations, the "gold standard" for the diagnosis of an AFR is an elimination diet trial followed by dietary challenges. Dietary selection, however, remains controversial. Single antigen, novel protein commercial diets can be unreliable due to contamination with foreign proteins.^{9,10} Hydrolysed diets are often effective for the diagnosis of cutaneous AFR but not completely reliable, possibly due to the presence of higher molecular weight, nonhydrolysed components.^{11–13} Some veterinarians recommend a home-prepared single novel antigen diet for the diagnosis of AFRs

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before evaluating balanced commercial diets for maintenance. The food allergens that most commonly elicit clinically relevant reactions in dogs are beef, dairy products, chicken, wheat, eggs, soy and lamb.^{14,15} Some dogs are multisensitive,¹⁶ either from co-sensitization or cross-reactivity. One study of 10 sera collected from dogs with confirmed AFRs demonstrated IgE reactivity to beef, lamb and milk antigens with antibodies that recognized bovine IgG in beef and cow's milk, and that cross-reacted with ovine IgG in lamb extract.⁵ Knowledge of allergenic cross-reactivity in foods could therefore be useful when assessing the likelihood of an AFR. Clearly, more extensive studies are required to identify potentially cross-reacting antigens in dogs with confirmed AFRs.

This study analysed food allergen-specific IgE reactivity in 469 sera from dogs with suspected allergic skin or gastrointestinal disease. Pairwise comparisons were undertaken to assess the significance of associations in the patterns of allergen recognition. Inhibition enzyme-linked immunosorbent assays (ELISAs) using sera reactive to beef, lamb and cow's milk were then performed to ascertain the prevalence and extent of cross-reactivity. The results could assist in the successful diagnosis and management of canine cutaneous AFRs.

Methods

Pairwise comparisons

Data from a commercial food allergen-specific IgE ELISA (Avacta Animal Health; Wetherby, UK) using serum from 469 dogs with suspected AFRs, submitted between January to April 2015, were evaluated retrospectively. Foods were categorized into taxonomically related groups: mammalian (beef, pork, lamb, venison, rabbit and cow's milk); avian (chicken, turkey, duck and whole hen's egg); fish (salmon and white fish); and plants (wheat, barley, soybean, potato, rice, corn and oat). Contingency tables for all the foods within each related group and between the foods from the unrelated groups were created to enable pairwise comparisons ($n = 171$) of positive (+) and negative (−) IgE reactions. The number of concordant results (+/+ or −/−) and discordant results (+/− or −/+) among food allergen pairs were used to calculate the odds ratios (ORs), 95% confidence intervals (CIs) and statistical significance. To control the false discovery rate, the level of significance was set at $P < 0.0002$ using a sequential Bonferroni technique^{17,18}. Pairwise tests were run both on all dogs ($n = 469$) and dogs with at least one positive IgE reaction ($n = 261$). The ORs for each food allergen pair were grouped into taxonomically related foods with a statistically significant association (associated-related foods), related foods with a nonstatistically significant association (nonassociated-related) and unrelated foods. The natural logarithm (base e) was used to transform the OR data into a normal distribution for further analysis.¹⁸ One-way ANOVA with Tukey's *post hoc* tests were used to test for differences between mean logE ORs in the associated-related, nonassociated-related and unrelated food groups. A level of $P < 0.05$ was considered significant. All analyses were performed using GraphPad Prism (GraphPad Software Inc.; San Diego, CA, USA).

ELISAs and inhibition ELISAs

Sera ($n = 115$) with a positive IgE reaction to at least two beef, lamb and cow's milk allergens were identified by ELISAs, performed as described previously.⁸ Briefly, beef, lamb and cow's milk extracts (Greer Labs Inc.; Lenoir, NC, USA) were coated at 5 µg/mL. Sera were assayed at a dilution of 1/10 and alkaline phosphatase (AP)-conjugated anti-dog IgE (clone 5.91, North Carolina State University; Raleigh, NC, USA) was used at 0.5 µg/mL. Plates were read on a

microplate reader (Tecan; Männedorf, Switzerland) at an absorbance of 405 nm (optical density, OD). A standard curve was prepared by assaying serial three-fold dilutions of a dog serum with high levels of beef-specific IgE, as determined by previous ELISAs. Undiluted, this serum was assigned a value of 500 arbitrary units (AU). After subtraction of the buffer-only (no-serum) control well ODs, mean standard ODs were fitted to a four-parameter sigmoidal standard curve (GraphPad Prism). Levels of specific IgE were determined from their mean duplicate ODs by interpolation from the resulting standard curve. A positive threshold of 5 AU was determined by interpolating the mean ODs + 3 standard deviations of *in vitro* allergen-specific IgE-negative sera (i.e. sera with ODs <0.25, 405 nm, to beef, lamb and cow's milk; $n = 124$) from the standard curve. Spearman's rank correlation was used to analyse AU levels for IgE binding to beef, lamb and cow's milk (GraphPad Prism).

Inhibition assays were performed using three related mammalian food allergens (beef, lamb and cow's milk) and one unrelated avian food allergen (turkey). To ensure that inhibitions were performed with comparable antibody titres, sera that gave ODs of 1.4 ± 0.7 (target OD $\pm$ allowed tolerance) upon 1/10 dilution were selected from previous noninhibited ELISAs. Sera ($n = 45$) were diluted 1/10 with 1, 5, 25, 625 and 3125 µg/mL solutions of beef, lamb, cow's milk and turkey extracts, and with assay buffer alone as a negative control. Serum/food allergen and serum/buffer mixtures were pre-incubated overnight at 2–8°C prior to measurement of IgE binding to beef, lamb and cow's milk allergen coats (all 5 µg/mL) by ELISA, as described above. After subtraction of buffer-only ODs, inhibition was calculated for each sample as follows: % inhibition = $100 - [(OD_{\text{serum with inhibitor}} / OD_{\text{serum without inhibitor}}) \times 100]$. The concentration of food allergen required to achieve 50% inhibition was interpolated from the sigmoid dose–response curves. Samples whose dose–response curves had an accuracy outside 80–120% of expected at concentrations effecting 50% inhibition were excluded from further analysis.

Food allergen concentrations for inhibition were determined from preliminary experiments using four dog sera, which showed that a concentration of up to 3125 µg/mL was required to effect maximum inhibition (>90%) on homologous coats.

Results

Incidence of reactions

Overall, 261 of 469 dogs (56%) had positive IgE reactions (i.e. >5 AU) to at least one of the 19 foods (Table 1). The frequency of positive IgE reactions to the 19 food allergens is shown in Figure 1. The median number of IgE food reactions per dog was four, with 26% of dogs reacting to a single food allergen and 74% reacting to two or more food allergens (Figure 2).

Pairwise comparisons

The results of pairwise analyses in all 469 dogs are given in Table 2. Pairwise comparisons were also used to calculate the ORs of the reactions to each food allergen pair in those dogs with at least one food IgE reaction (positive reactors; Table 3).

All dogs (n = 469)

Table 1. Incidence of IgE reactions to food allergens

IgE reaction	Number of dogs ($n = 469$)	% dogs
Positive to at least one food	261	56%
Positive to more than one food	194	41%
Negative to all foods	208	44%

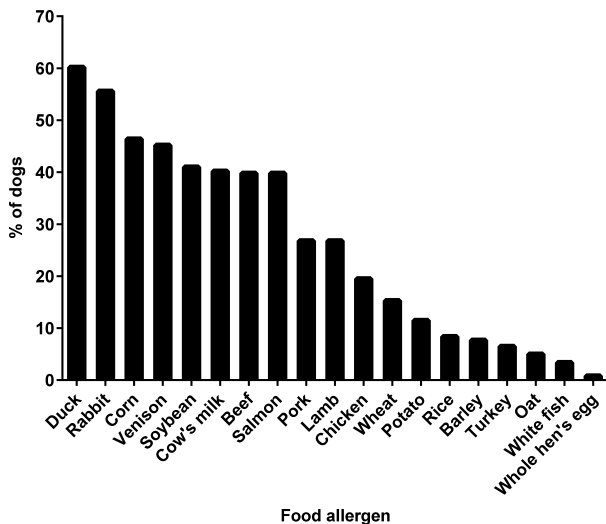


Figure 1. Percentage frequency of serum IgE reactions to food allergens.

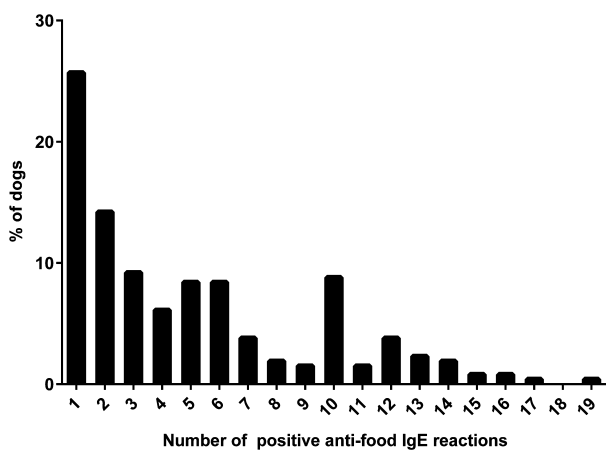


Figure 2. The frequency of IgE reactions to one or more food allergens (range 1–19 allergens) in 469 dogs with suspected adverse food reactions (AFRs).

With the exception of egg, white fish and potato, at least 50% of the comparisons for each allergen were significantly associated, with mean logE ORs ranging from 1.3 (CI 0.6–3.0) to 215.3 (CI 27.8–1668); $P = 0.6$ to $P < 0.0001$ (Table 2). In these dogs, 38 of 43 related food pairs were significantly associated compared with 79 of 128 unrelated pairs ($P < 0.0002$). The mean (SD) logE OR of these associated-related pairs [3.4 (0.9)] was significantly greater than unrelated pairs [2.7 (1.0)], $P < 0.05$ (Table 4).

Positive reactors ($n = 261$)

For all foods except egg, white fish, potato, rabbit, duck, turkey, barley, rice and oats, at least 50% of the comparisons for each allergen were significantly associated, with mean logE ORs ranging from 0.5 (CI 0.2–1.1) to 213.6 (CI 12.5–3636); $P = 1.0$ to $P < 0.0001$ (Table 3). Of the related allergen pairs, 32 of 43 were significantly associated compared with 49 of 128 unrelated pairs ($P < 0.0002$). The mean (SD) logE OR of these associated-related pairs [2.7 (1.0)] was significantly greater than

all unrelated pairs [1.8 (1.0)], $P < 0.05$ (Table 4) and unrelated pairs with a statistically significant association [2.3 (0.8)], $P = 0.035$.

ELISAs and inhibition ELISAs

ELISA AU scores to beef and lamb were very strongly correlated ($r = 0.93$); 75 of 90 (83.3%) beef-positive sera were also lamb-positive and 75 of 81 (92.6%) of lamb-positive sera were positive to beef. AUs to lamb and milk were also strongly correlated ($r = 0.62$); 57 of 81 (70.4%) of lamb-positive sera were milk-positive and 57 of 85 (67.1%) milk-positive sera were positive to lamb. A moderate correlation between beef and milk was observed ($r = 0.59$); 62 of 90 (68.9%) beef-positive sera were also positive to milk and 62 of 85 (72.9%) of milk-positive sera were positive to beef.

Pre-incubation of sera with homologous allergen inhibitor resulted in almost complete (>88%) inhibition at the maximal inhibitory concentration of 3125 $\mu\text{g/mL}$. The mean concentration of inhibitor required for 50% inhibition of IgE binding to homologous allergen coats was 5.6 $\mu\text{g/mL}$ (range <1–60.2 $\mu\text{g/mL}$), 27.8 $\mu\text{g/mL}$ (range <1–371.7 $\mu\text{g/mL}$) and 5.3 $\mu\text{g/mL}$ (range <1–34.4 $\mu\text{g/mL}$) for beef, lamb and milk, respectively.

Pre-incubation of sera with beef, lamb and milk resulted in 50% inhibition of IgE binding to mammalian protein substrates in the majority of sera (Figure 3). With beef as the coat, 50% inhibition was achieved in 27 of 27 sera by pre-incubation with lamb (mean concentration required for 50% inhibition 457.7 $\mu\text{g/mL}$, range 26–2016.4 $\mu\text{g/mL}$) and in 16 of 20 sera by milk (mean concentration required for 50% inhibition 666.5 $\mu\text{g/mL}$, range 1–2998.7 $\mu\text{g/mL}$). With lamb as the coat, 50% inhibition was effected in 19 of 19 sera by beef (mean concentration required for 50% inhibition 262.3 $\mu\text{g/mL}$, range 1–1786.6 $\mu\text{g/mL}$) and in 17 of 23 sera by milk (mean concentration required for 50% inhibition 419.3 $\mu\text{g/mL}$, range 1–1427 $\mu\text{g/mL}$). With milk as the coat, 10 of 19 sera were inhibited by lamb (mean concentration required for 50% inhibition 883.5 $\mu\text{g/mL}$, range 1–2785.1 $\mu\text{g/mL}$) and 14 of 16 by beef (mean concentration for 50% inhibition 616.4 $\mu\text{g/mL}$, range 1–2837.4 $\mu\text{g/mL}$). In contrast, pre-incubation of sera with turkey effected little to no inhibition of IgE binding to beef, lamb and cow's milk coats.

Discussion

In this study, we investigated whether serum IgE reactions were associated more frequently among related food allergens than unrelated food allergens, and whether such associations were the result of cross-reactivity. Pair-wise comparisons showed that dogs with a positive IgE reaction to one food within a related group were significantly more likely to have a positive IgE reaction to another food within the same group than to an unrelated food. Moreover, IgE scores to beef, lamb and cow's milk were strongly correlated. However, another approach, using ELISA inhibition, was necessary to ascertain if associations between related food allergens were the result of immunological cross-reactivity.

Although the majority of the 469 dogs were positive to at least one food allergen, approximately 40% of the dogs

Table 2. Odds ratios (ORs, 95% confidence intervals) of food allergen pairs in all dogs ($n = 469$ dogs).

	Pork	Lamb	Venison	Rabbit	Milk	Chicken	Turkey	Duck	Egg	Salmon	White fish	Wheat	Soybean	Barley	Rice	Potato	Corn	Oat
Beef	32.8* (16.7–64.5)	11.3* (6.4–19.9)	19.4* (11.4–33.0)	13.3* (8.0–22.4)	9.1* (5.5–14.9)	12.3* (6.4–23.0)	18.8* (5.3–66.7)	45.0* (22.0–91.0)	17.8 (0.8–374.0)	34.7* (18.0–66.0)	30.4 (3.7–245.0)	5.8* (3.0–11.4)	8.1* (5.0–13.3)	5.8 (2.3–14.7)	3.8 (1.6–9.1)	2.5 (1.2–5.4)	5.1* (3.2–8.1)	3.1 (1.0–9.5)
Pork		30.0* (15.7–57.0)	31.0* (15.3–62.3)	142.2* (34.1–592.9)	15.6* (8.7–28.3)	29.0* (14.4–59.5)	54.1* (12.1–243.0)	243.0* (33.0–1780.0)	29.0 (1.3–614.0)	32.8* (16.7–123.0)	123.0 (7.1–2147.0)	12.5* (6.2–25.0)	10.6* (6.1–19.0)	16.4* (6.1–44.0)	12.2* (5.2–21.0)	3.2 (1.4–7.0)	18.5* (9.9–34.0)	37.0* (8.1–171.0)
Lamb			40.1* (19.1–86.4)	142.2* (34.1–593.0)	14.3* (8.0–25.6)	65.0* (13.0–146.0)	54.0* (12.0–243.0)	98.0* (30.0–320.0)	29.0 (1.4–614.0)	48.0* (23.0–101.0)	109.0 (6.3–1898.0)	8.5* (4.3–17.0)	11.5* (6.5–20.0)	10.1* (3.9–25.0)	5.3 (2.2–13.0)	2.7 (0.9–5.1)	13.8* (7.6–25.0)	10.1 (3.2–32.0)
Venison				29.2* (16.6–51.1)	13.2* (8.0–21.9)	29.0* (13.0–67.0)	25.0* (5.7–113.0)	26.0* (15.0–46.0)	15.0 (0.7–316.0)	26.0* (15.0–46.0)	61.0 (3.5–1056.0)	8.7* (4.3–18.0)	10.2* (6.3–16.7)	7.7* (2.9–20.0)	4.7 (1.9–11.3)	1.3 (0.6–3.0)	9.6* (5.9–15.0)	7.1 (2.1–23.0)
Rabbit					9.3* (5.7–15.1)	38.0* (13.0–109.0)	40.0 (5.2–305.0)	27.0* (16.0–46.0)	11.3 (0.5–237.0)	29.0* (16.0–54.0)	45.0 (2.6–781.0)	11.0* (5.1–25.0)	10.4* (6.3–17.0)	7.3 (2.6–21.0)	6.5* (2.5–17.0)	2.1 (1.1–4.5)	7.6* (4.8–12.0)	29.0 (226.0)
Milk						22.0* (11.1–23.0)	30.0* (6.7–134.0)	23.0* (11.2–48.0)	3.5 (0.2–56.0)	15.5* (9.2–27.0)	71.0 (4.1–1244.0)	10.1* (5.2–21.0)	12.4* (7.5–33.0)	8.5* (4.1–21.0)	1.5 (0.7–3.4)	13.1* (7.9–21.0)	21.0* (9.7.0)	
Chicken							215.3* (27.8–1668.0)	25.5* (9.9–65.6)	17.0 (1.5–191.0)	91.0* (27.0–303.0)	77.0* (9.5–635.0)	6.6* (3.2–25.0)	13.2* (6.7–31.0)	8.0* (3.1–20.0)	6.8* (2.7–16.0)	2.7 (1.1–6.7)	23.4* (10.6–52.0)	15.4* (4.8–49.0)
Turkey								35.3* (4.6–268.0)	60.1* (5.2–700)	66.0* (8.7–505.0)	47.0* (11.1–195.0)	15.2* (5.5–42.0)	29.3* (6.6–130.0)	17.0* (5.5–52.0)	7.4 (2.2–25.0)	2.0 (0.4–9.2)	52.0* (6.9–403.0)	23.0* (8.1.0)
Duck									4.0 (0.4–44.6)	39.0* (20.0–78.0)	40.0 (2.3–691.0)	11.6* (5.0–27.0)	12.5* (7.5–21.0)	8.7* (2.9–26.0)	7.5* (2.7–20.0)	2.8 (1.3–5.9)	9.0* (5.7–14.0)	25.0 (3.3–199.0)
Egg										17.8 (0.8–374.0)	57.0 (3.3–1000.0)	10.9 (0.7–178.0)	3.4 (0.2–55.0)	23.5 (1.4–391.0)	21.2 (1.3–351.0)	15.0 (0.9–247.0)	14.6 (0.7–305.0)	37.9 (2.2–642.0)
Salmon											34.5* (4.3–275.0)	10.8* (5.3–22.0)	19.7* (11.5–33.0)	7.3* (2.8–16.0)	5.7* (2.3–13.6)	1.8 (0.8–4.0)	11.8* (7.2–19.0)	12.8 (4.7.0)
White fish												15.0* (3.9–59.0)	70.0 (4.2–1211.0)	22.2* (38.0–91.0)	11.6 (7.1–50.0)	1.9 (0.2–15.0)	58.0 (1019.0)	40.0* (174.0)
Wheat													45.4* (15.6–131)	174.0* (38.0–800.0)	18.0* (7.1–45.0)	16.6* (7.3–105.0)	36.4* (12.6–181.0)	47.3* (12.4–181.0)
Soybean														22.6* (6.5–78.8)	8.3* (3.3–20.9)	3.3 (1.5–9.9)	16.1* (9.6–27.0)	20.6* (4.5–94.6)
Barley															90.8* (29.3–281.0)	35.9* (13.0–98.0)	140.0 (8.4–2347.0)	148.0* (35.0–623.0)
Rice																17.8* (6.8–46.0)	34.0* (20.1–149.0)	77.0* (281.0)
Potato																	9.4* (4.1–21.0)	15.4* (4.8–49.0)
Corn																		86.7 (5.1–1170.0)

*Significantly associated ($P < 0.0002$).

had no IgE reaction to any of the 19 foods tested. None of the dogs in this study had a definitive diagnosis of AFR. Therefore, we do not know if these dogs were suffering from conditions other than an AFR, had non-IgE-mediated AFRs, or had AFRs to food(s) other than those included here. To eliminate the possibility that these dogs could have skewed the results, pairwise comparisons were repeated on data from dogs with at least one positive IgE reaction. The overall outcome was the same: a significant association was more likely between related food pairs than unrelated foods.

Significant associations between unrelated food allergens were observed frequently. However, it is notable that ORs for these associated-unrelated foods were significantly lower than associated-related foods. Pairwise

analyses do not differentiate between cross-reactivity and co-sensitization, and we did not confirm the nature of these associations by ELISA inhibition. Having ascertained that significant associations existed between related foods, the presence of serum IgE antibodies cross-reacting with different mammalian species was assessed by inhibition ELISAs. The food extracts were used at concentrations over 600 times greater than those used for coating, thus effecting maximum homologous inhibition of IgE binding and ensuring that any cross-reactive IgE antibodies with low affinity for an extract could be identified. Considerable heterologous inhibition of IgE binding was observed with beef and lamb. Milk had less effect on IgE binding to beef and lamb, with fewer dogs reaching 50% inhibition, even at high inhibitor

Table 3. Odds ratios (ORs, 95% confidence intervals) of food allergen pairs in positive reactors ($n = 261$ dogs)

	Pork	Lamb	Venison	Rabbit	Milk	Chicken	Turkey	Duck	Egg	Salmon	White fish	Wheat	Soybean	Barley	Rice	Potato	Corn	Oat	
Beef	13.4* (6.8–26.7)	4.4* (2.5–7.9)	6.9* (3.9–11.9)	4.03* (2.3–6.9)	3.2* (1.9–5.3)	5.0* (2.6–9.8)	8.0 (2.2–28.6)	14.0* (6.8–29.0)	7.7 (0.4–161.8)	12.5* (6.4–24.3)	13 (1.6–105.6)	2.3 (1.2–4.6)	2.8* (1.7–4.6)	2.4 (1.0–6.2)	1.6 (0.7–3.8)	1.0 (0.5–2.2)	1.5 (0.9–2.5)	1.3 (0.4–4.0)	
Pork		13.3* (6.9–25.6)	12.0* (5.9–24.5)	50.3* (12.0–211.6)	6.2* (3.4–11.3)	14.2* (6.9–29.1)	25.8* (5.7–116.2)	80.8* (11.0–593.8)	14.0 (0.7–295.0)	13.4* (6.8–26.7)	59.2* (3.4–1032)	5.7* (2.8–11.6)	4.1* (2.3–7.3)	7.7* (2.8–21.0)	5.7* (2.3–14.3)	1.4 (0.6–3.2)	6.9* (3.6–13.2)	17.6* (3.8–81.8)	
Lamb			15.9* (7.4–34.3)	50.3* (12.0–211.6)	5.6* (3.1–10.2)	30.3* (13.3–69.1)	25.8* (5.7–116.2)	25.1* (7.6–82.5)	14.0 (0.7–295.0)	20.1* (9.5–42.4)	59.2 (3.4–1032)	3.9 (1.9–7.8)	4.5* (2.5–8.0)	4.7 (1.8–12.1)	2.5 (1.0–6.0)	1.0 (0.4–2.3)	5.1* (2.8–9.5)	4.8 (1.5–15.2)	
Venison				9.1* (5.1–16.4)	4.5* (2.7–7.6)	11.6* (5.0–26.9)	10.3 (2.3–45.9)	7.6* (4.2–13.8)	6.2 (0.3–129.6)	9.6* (5.4–17.0)	24.9 (1.4–432.8)	3.4 (1.6–7.0)	3.4* (2.0–5.7)	3.1 (1.1–8.3)	1.8 (0.8–4.5)	0.5 (0.2–1.1)	3.1 (1.9–5.0)	2.9 (0.9–9.6)	
Rabbit					2.7 (1.6–4.5)	13.4* (4.7–38.6)	14.3 (1.9–109.3)	7.3* (4.2–12.8)	4.1 (0.2–85.5)	9.6* (5.2–18.0)	16.2 (0.9–281.8)	3.8 (1.7–8.7)	3.0* (1.8–5.1)	2.6 (0.9–7.3)	2.3 (0.9–6.0)	0.7 (0.3–1.4)	2.0 (1.2–3.3)	10.4 (1.3–81.1)	
Milk						9.4* (4.4–19.8)	12.8* (2.9–57.5)	2.8 (1.6–4.8)	1.5 (0.1–24.1)	5.7* (3.3–9.8)	30.9 (1.7–535.8)	4.4* (2.1–9.1)	4.4* (2.6–7.5)	5.0 (1.8–14.3)	3.5 (1.4–9.0)	0.6 (0.3–1.0)	4.4* (2.6–7.5)	9.0 (2.0–41.6)	
Chicken							213.6* (12.5–3636)	8.2* (3.1–21.5)	21.3 (1.0–450.3)	19.2* (7.8–47.4)	38.9* (4.7–319.2)*	3.1 (1.5–6.4)	5.3* (2.9–10.4)	3.9 (1.5–9.9)	3.2 (1.3–8.1)	1.3 (0.5–3.2)	9.1* (4.1–20.4)	7.6 (2.4–24.5)	
Turkey								11.7 (1.5–89.6)	78.9 (3.6–1716)	28.4* (3.7–217.6)	25.0* (5.9–105.2)	7.7 (2.8–21.5)	12.4* (2.8–55.4)	9.0 (2.9–27.8)	3.9 (1.1–13.1)	1.0 (0.2–4.7)	21.2* (2.8–162.3)	12.3 (3.5–43.3)	
Duck									3.4 (0.2–70.8)	12.3* (6.1–24.8)	13.4 (0.8–232.4)	3.7 (1.6–8.7)	3.5* (2.0–6.0)	2.8 (0.9–8.7)	2.4 (0.9–6.7)	0.9 (0.4–1.8)	2.2 (1.3–3.7)	8.5 (1.1–66.6)	
Egg										7.7 (0.4–161.8)	31.4 (1.8–548.1)	5.6 (0.3–92.2)	1.4 (0.1–23.4)	12.6 (0.8–210.1)	11.3 (0.7–187.9)	7.9 (0.5–130.3)	5.9 (0.3–123.7)	20.6 (1.2–349.6)	
Salmon											31.3 (1.8–544.9)	4.5* (2.1–9.2)	7.2* (4.1–12.6)	3.1 (1.2–8.0)	2.3 (1.0–5.7)	0.7 (0.3–1.6)	4.0* (2.4–6.7)	5.5 (1.5–20.4)	
White fish												7.8 (2.0–30.3)	29.8 (1.7–518.2)	11.8 (2.9–48.3)	6.1 (1.4–26.5)	1.0 (0.1–8.0)	23.7 (1.4–412.4)	21.6 (4.9–94.3)	
Wheat														19.0* (6.5–55.5)	89.6* (19.5–411.9)	9.0* (3.6–22.9)	8.2* (3.6–18.9)	14.4* (4.9–41.9)	24.2* (6.3–93.0)
Soybean															9.5* (2.7–33.4)	3.4 (1.3–8.7)	1.3 (0.6–2.8)	5.5* (3.2–9.4)*	8.8 (1.9–40.2)
Barley																47.9* (15.4–148.9)	18.6* (6.7–51.3)	56.8* (3.4–950.0)	79.3* (18.9–334.0)
Rice																	9.1* (3.5–23.7)	13.7* (3.1–59.84)	40.7* (11.0–149.8)
Potato																		8.0 (1.6–8.6)	25.8 (2.5–25.8)
Corn																		35.0* (2.1–595.1)	

*Significantly associated ($P < 0.0002$).**Table 4.** LogE odds ratios (ORs) in all dogs and dogs with at least one positive reaction (positive reactors)

	LogE ORs					
	All dogs (469 dogs)			Positive reactors (261 dogs)		
	Associated-related	Nonassociated-related	Unrelated	Associated-related	Nonassociated-related	Unrelated
Mean	3.4*	3.0	2.7*	2.7†	2.1	1.8†
SD	0.9	1.7	1.0	1.0	1.2	1.0
n	38	5	128	32	11	128

SD, standard deviation; n, number of pairwise comparisons.

*Mean logE ORs of associated-related food pairs and unrelated food pairs that were significantly different in all dogs. ($P < 0.05$).†Mean logE ORs of associated-related foods and unrelated foods that were significantly different in positive reactors. ($P < 0.05$). Mean logE ORs of associated-related food pairs and nonassociated-related or unrelated food pairs were not statistically different ($P > 0.05$).

concentrations. Furthermore, higher mean concentrations of beef and lamb were required to achieve 50% inhibition of IgE binding to milk. Lower heterologous inhibition of IgE binding to beef and lamb by milk could be due to a lack of cross-reactive proteins in the milk extract and/or lower IgE binding affinity to milk proteins

compared with beef and lamb. Inhibition data were thus consistent with the presence of cross-reactive IgE binding epitopes in beef and lamb, and to a lesser extent, cow's milk. These IgE binding epitopes were largely absent in turkey. Previous studies have identified a number of common mammalian food allergens. In humans,

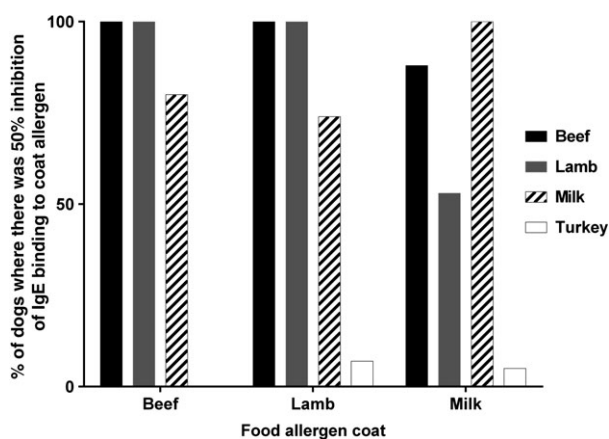


Figure 3. Percentage of dogs where there was 50% inhibition of IgE binding to beef, lamb and cow's milk allergen coats (5 µg/mL) following preincubation of sera with beef, lamb, cow's milk and turkey inhibitors (3125 µg/mL).

various allergenic beef proteins have been identified including bovine serum albumin (BSA, Bos d 6), actin and IgG (Bos d 7).¹⁹ IgG and muscle phosphoglucosyltransferase were also found to be major cross-reactive allergens in dogs with specific IgE against beef, cow's milk and, importantly, lamb,⁵ which was, until fairly recently, commonly used in limited antigen diets.

The likelihood of cross-reactivity is increased amongst closely related foods, particularly if amino acid sequence homology is greater than 70%.²⁰ Beef, lamb and cow's milk are derived from the same biological family (Bovidae) and share a recent common ancestor. As a consequence they are more likely to have similar antigens, leading to increased cross-reactivity. In contrast, turkey shared a common ancestor with mammals more than 100 million years ago and cross-reactivity with mammalian proteins is unlikely. Cross-reactivity has, however, been described between phylogenetically distinct species,²¹ particularly among highly conserved proteins. For example, serum albumins have highly conserved amino acid sequences and three-dimensional structures amongst a diverse range of species. Turkey serum albumin displays moderate (~50%) sequence identity and similarity to BSA,²² although this is below the 70% identity threshold believed to be required for immunological cross-reactivity. In humans, the clinical significance of allergenic cross-reactivity has been variable. A number of studies in patients with cow's milk allergy demonstrated that between 60 and 87.5% had specific IgE to beef.^{23,24} However, a review of studies in infants with cow's milk allergy found that only 13–20% were also beef allergic; interestingly, in their own double-blind, placebo-controlled food challenge study, the authors found up to 92.9% of children with beef allergy had concomitant milk allergy.²⁵ It is not known how many dogs in the present study had confirmed cutaneous AFR. Therefore, despite the demonstration of shared IgE-binding epitopes between related foods in some of the dogs, their clinical relevance is unknown.

In conclusion, this study demonstrated that associations in IgE reactivity between the related mammalian

foods beef, lamb and cow's milk were, in some cases, due to the presence of cross-reactive epitopes. The observation of single IgE reactions to an allergen within a related food group known to share IgE-binding epitopes, however, suggests that there may be considerable heterogeneity in food-specific IgE reactions among dogs and that extrapolating cross-reactivity data to the dietary management of AFRs is not straightforward. ELISAs for food allergen-specific IgE could be helpful in identifying potential allergenic reactivity against novel proteins, and have been used to guide the selection of foods for inclusion in elimination diet trials based on a lack of IgE reaction to those foods.⁸ Such tests cannot, however, definitively predict a clinical reaction or exclude the possibility that novel proteins could induce an allergic reaction in susceptible individuals. Thus, although it would be prudent to avoid cross-reactive and/or closely related foods in the selection of foods for elimination diet trials, further research is required to determine which, if any, cross-reactive food allergens mediate clinically relevant reactions in allergic dogs.

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Résumé

Contexte – La connaissance sur la réactivité croisée entre les aliments est utile afin de déterminer quels allergènes doivent être évités dans les régimes d'éviction.

Hypothèses/Objectifs – Évaluer la réactivité croisée allergénique des aliments.

Sujets – Les sera de 469 chiens suspectés de réactions alimentaires indésirables.

Méthodes – Un dosage sérologique basé sur les IgE utilisant 19 allergènes alimentaires a été réalisé chez 469 chiens. Des comparaisons par paire ont été utilisées pour calculer l'odd ratio (ORs) de chaque paire, avec valeur significative à une correction Holm-Bonferroni $P < 0.0002$, pour les 469 chiens et les 261 des 469 chez avec au moins une réaction positive. Des tests ANOVA à sens unique post-hoc de Turkey (significatif à $P < 0.05$) ont été utilisés pour tester les différences entre les ORs logE moyens des différents groupes d'aliments. Des tests ELISA (inhibition enzyme-linked immunosorbent assays) ont été réalisés pour évaluer la réactivité croisée allergénique entre le bœuf, l'agneau et le lait de vache.

Résultats – Les associations significatives ont été observées entre les paires d'aliments à la fois reliés et non-reliés. Les associations étaient cependant, plus fréquentes et fortes parmi les aliments reliés que non-reliés. Pour tous les 469 chiens, 38 des 43 paires d'aliments reliés étaient significativement associés [moyenne (SD) logE OR 3.4 (0.9)] comparés avec 79 des 128 paires non-reliées [2.7 (1.0)], $P < 0.0002$. Chez les chiens positifs, 32 des 43 paires reliées étaient significativement associées [2.7 (1.0)] comparé aux 49 des 128 paires non reliées [1.8 (1.0)], $P < 0.0002$. Les ELISA ont confirmé la présence de réactivité croisée des épitopes porteurs D'IgE du bœuf, agneau et lait de vache.

Conclusions et importance clinique – Ces résultats suggèrent que les aliments reliés et à réactivité croisée doivent être évités dans les tests d'éviction.

Resumen

Introducción – el conocimiento de la reactividad cruzada entre alimentos es útil para evitar el uso de alérgenos con reacciones cruzadas en las pruebas de supresión alimentaria.

Hipótesis/Objetivos – evaluar la reactividad cruzada de alimentos relacionados.

Animales – suero de 469 perros con sospecha de reacciones adversas alimentarias.

Métodos – un ensayo basado en la detección de IgE en suero utilizando 19 alérgenos alimentarios fue desarrollada en los 469 perros. Se utilizó una comparación pareada para calcular la razón de probabilidades (ORs) para cada par de alimentos, con significación estadística a $P < 0.0002$ con la corrección de Holm-Bonferroni, en todos los pacientes los 469 perros y en los 261 perros de los 469 con al menos una reacción positiva. Se utilizó análisis de varianza con prueba de medias de Tukey (significación estadística a $P < 0.05$) para diferenciar entre la media del logaritmo neperiano de las medias de probabilidades de los diferentes grupos de alimentos. Una prueba de inmunoabsorción asociado a enzimas (ELISA) fue desarrollada para evaluar la reactividad cruzada entre alérgenos en carne de vacuno, cordero y leche de vaca.

Resultados – se observó una asociación significativa entre parejas de alimentos relacionados y no relacionados. Las asociaciones fueron sin embargo más frecuentes y más fuertes entre alimentos relacionados que no relacionados. En todos los 469 perros, 38 de 43 pares de alimentos relacionados estuvieron significativamente asociados (media (SD) del logaritmo neperiano OR 3,4 (0,9)) comparados con 79 de 128 parejas no relacionadas (2,7 (1,0)), $P < 0.0002$. En perros positivos, 32 de 43 parejas relacionadas estuvieron significativamente asociadas (2,7 (1,0)) comparados con 49 de 128 parejas no relacionadas (1,8 (1,0)), $P < 0.0002$. La prueba de Elisa por inhibición confirmó la presencia de reactividad cruzada entre epítomos de unión a IgE entre carne de vacuno, cordero y leche de vaca.

Conclusión e importancia clínica – los resultados sugieren que alimentos relacionados y potencialmente con reactividad cruzada deben ser eliminados en las pruebas de eliminación dietética.

Zusammenfassung

Hintergrund – Das Wissen über Kreuzreaktionen bei Futtermitteln ist wichtig, um mögliche Kreuz-reaktive Allergene bei Eliminationsdiäten auszuschließen.

Hypothese/Ziele – Eine Evaluierung der Kreuz-Reaktivität von Allergenen in verwandten Futtermitteln.

Tiere – Es wurden 469 Sera von Hunden mit Verdacht auf Reaktionen auf Futtermittel untersucht.

Methoden – Ein auf IgE-basierender serologischer Assay, der 19 Futterallergene verwendet, wurde bei 469 Hunden angewandt. Es wurden paarweise Vergleichsuntersuchungen durchgeführt, um das Quotenverhältnis/ Odds Ratio (ORs) für jedes Futterpaar, bei einer Signifikanz von $P < 0,0002$ mittels Holm–Bonferroni Korrektur, sowohl bei allen 469 Hunden wie auch bei den 261 der 469 Hunde mit mindestens einer positiven Reaktion, zu kalkulieren. Die Einweg Varianzanalyse mit Tukey Test (Signifikanz $P < 0,05$) wurde eingesetzt, um Unterschiede zwischen den durchschnittlichen logE ORs in den verschiedenen Futtermittelgruppen zu untersuchen. Inhibitions Enzyme-linked Immunosorbent Assays (ELISAs) wurden durchgeführt, um die Kreuzreaktivität der Allergene zwischen Rind, Lamm und Kuhmilch zu untersuchen.

Ergebnisse – Es wurden signifikante Zusammenhänge zwischen sowohl verwandten wie auch nicht verwandten Futtermittelpaaren beobachtet. Die Zusammenhänge waren jedoch zwischen den verwandten häufiger und stärker als bei den nicht verwandten Futtermittelpaaren. Bei allen 469 Hunden standen 38 von 43 verwandten Futtermittelpaaren signifikant im Zusammenhang [durchschnittliche (SD) logE oR 3,4 (0,9)] im Vergleich zu den 79 der 128 nicht zusammenhängenden Paare [2,7 (1,0)], $P < 0,0002$. Bei positiven Hunden waren 32 der 43 verwandten Paare signifikant assoziiert [2,7(1,0)] im Vergleich zu 49 der 128 nicht verwandten Paare [1,8 (1,0)], $P < 0,0002$. Inhibitions ELISAs bestätigten das Vorkommen von Kreuz-reaktiven IgE bindenden Epitopen bei Rind, Lamm und Kuhmilch.

Schlussfolgerungen und klinische Bedeutung – Die Ergebnisse weisen darauf hin, dass verwandte und möglicherweise Kreuz-reaktive Futtermittel bei Eliminationsdiäten vermieden werden sollten.

要約

背景 – 食物間の交差反応に関する情報は、食餌療法において交差反応する可能性のあるアレルゲンを回避できることから有益である。

仮説/目的 – 関連する食物におけるアレルゲン交差反応性を評価すること。

供与動物 – 食物有害反応が疑われた469頭のイヌの血清。

方法 – 19の食物アレルゲンをを用いたIgEに基づく血清学的分析を469頭のイヌで行った。すべての469頭のイヌと少なくとも1つ以上の陽性反応を示した469頭中261頭で比較するそれぞれの食物間において、オッズ比(ORs)計算のために対比較を使用し、ホルム–ボンフェローニ補正により $P < 0.0002$ を有意差とした。Tukeyのポストホックテストを用いた一元ANOVA(有意差 $P < 0.05$)を、異なる食品群の平均logE ORs間の差を評価するために使用した。阻害酵素結合免疫吸着法(ELISAs)を牛肉、ラム肉および牛乳の間のアレルゲン交差反応を評価するために実施した。

結果 – 関連する食物内と関連していない食物間の両方で有意な関連性が観察された。しかし、相関は関連しない食物と比較して関連する食物の間でより頻度が高く、より強く認められた。469頭のイヌ全体において、 $P < 0.0002$ として、関連しない食物間の陽性反応の238の組み合わせのうち79 [1.8 (1.0)]で有意な相関を示していたのと比較し、関連する食物内の陽性反応の43の組み合わせのうち38[平均(SD) logE OR 3.4 (0.9)]が有意な相関を示していた。陽性のイヌにおいて、 $P < 0.0002$ として、関連しない食物間の陽性反応の128の組み合わせのうち49で有意な相関を示していた[1.8 (1.0)]のに対して、関連する食物内の陽性反応の43の組み合わせのうち32ペアで有意な相関を示していた[2.7 (1.0)]。阻害ELISAsにより牛肉、ラム肉および牛乳において交差反応IgE結合エпитープの存在が確認された。

結論および臨床的な重要性 – 今回の結果は関連する食物内および可能性のある交差反応食物は、除去食試験において回避すべきであることを示唆している。

摘要

背景 – 了解食物间的交叉反应非常有意义,在选择食物时可避免潜在的交叉过敏反应。

假设/目的 – 评估相关食物的交叉过敏反应。

动物 – 取469只疑似食物过敏反应患犬血清。

方法 – 在469只犬上用19种食物过敏原进行IgE为基础的血清学评估。每组食物计算比例(ORs)成对对比,使用邦费罗尼校正法 $P < 0.0002$ 非常显著,无论是469只犬或是469只中的261只犬均至少有一种阳性过敏反应。单向分析方差后,进行Tukey's事后检验法($P < 0.05$ 显著)探测不同食物logE ORs平均值的不同。通过抑制酶联免疫吸附试验(ELISAs),评估牛羊肉和牛奶间的交叉过敏反应。

结果 – 发现有关联和无关联的食物组合均有明显相关性。有关联食物和无关联食物相比,相关性更强和更密切。在469只犬中,43组有关联食物组合中的38组具有明显相关性[平均 (SD) logE OR 3.4 (0.9)],128组无关联食物组合中,有79组具有明显相关性[2.7 (1.0)], $P < 0.0002$ 。阳性犬中,43组有关联食物组合中的32组具有明显相关性[2.7 (1.0)],128组无关联食物组合中,有49组具有明显相关性[1.8 (1.0)], $P < 0.0002$ 。ELISAs抑制试验可确定牛肉、羊肉和牛奶存在交叉反应IgE结合表型。

总结和临床意义 – 结果显示,在进行食物排除试验时,应避免接触有相关性和具有潜在交叉反应的食物。