

Identification of allergens responsible for canine cutaneous adverse food reactions to lamb, beef and cow's milk

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Abstract Lamb, beef and cow's milk are common causes of cutaneous adverse food reactions in dogs. The aim of this study was to identify the proteins responsible for cutaneous adverse reactions to these foods. Ten dogs with allergen-specific serum immunoglobulin (Ig)E to lamb, beef and cow's milk were included in the study. These dogs had been diagnosed with cutaneous adverse food reactions by convincing clinical history and food-elimination diet trials followed by challenge exposure. Sera were analysed by enzyme-linked immunosorbent assay with bovine proteins and SDS-PAGE immunoblots with lamb, beef and cow's milk extracts. All the dogs had specific IgE against bovine IgG, and it was the only protein in the cow's milk extract that bound IgE from the sera studied. In the lamb and beef extracts, the major allergens recognized by the specific IgE of most sera had molecular masses between 51 and 58 kDa, which were identified as phosphoglucosyltransferase and the IgG heavy chain. Other IgE-binding proteins with molecular masses of 27, 31, 33, 37 and 42 kDa were also detected with some sera. Our results indicate that bovine IgG is a major allergen in cow's milk and hence it appears to be a source of cross-reactivity with beef and probably with lamb because of the high homology with ovine immunoglobulins. These results are similar to those found for meat allergy in humans. However, this is the first time that phosphoglucosyltransferase has been identified as an important allergen involved in allergic reactions to lamb and beef.

Keywords: allergen, beef, cow's milk, cutaneous adverse food reaction, food allergy, lamb.

INTRODUCTION

About 1% of all dogs and cats experience adverse reactions to ingested foods that can produce symptoms involving the skin, gastrointestinal tract, respiratory tract and central nervous system.¹ In dogs, the incidence of cutaneous adverse food reactions (CAFR) is estimated at 1–5% of all skin conditions and up to 23% of cases of nonseasonal allergic dermatitis.² In humans, adverse food reactions may be caused by nonimmunological (food intolerance) or immunological (food allergy) phenomena, which can be elicited through either a cellular or immunoglobulin (Ig)E-mediated mechanism. It is known that most food allergic reactions in humans are IgE-mediated,³ and diagnostic decision points for allergen-specific serum IgE concentrations have been described for some foods, such as cow's milk.⁴ In contrast, the percentage of allergic reactions to foods that are mediated by IgE in dogs has not yet been established. Furthermore, the pathological mechanisms of CAFR in dogs have not been fully elucidated and, accordingly, the ACVD task force recommends the use of this broader term instead of food allergy, irrespective of the underlying mechanism.⁵

In principle, any food has the potential to induce adverse reactions, although only a limited number of ingredients have been identified as probable causes. These ingredients vary depending on the dietary habits in different countries; those with the highest protein content and consumption usually being the most relevant CAFR-eliciting agents.⁶ In western countries, meat of mammalian origin and products derived from cow's milk are among the main sources of protein in the diets of carnivore pets, and sensitization to them has been reported in a high proportion of dogs and cats suffering from CAFR. In a Canadian study performed with a large number of animals (86 cases), beef and dairy products were the most prevalent foods responsible for CAFR.⁷ Likewise, in two studies carried out in the USA,^{8,9} these foods, along with soybean, were the most commonly encountered causes of CAFR. In France, Carlotti *et al.*¹⁰ also found that beef and dairy products most often caused CAFR. Lamb used to be habitually included in elimination diets for beef-allergic animals, however, cases of CAFR to this meat have also been reported.^{11,12}

Using an enzyme-linked immunosorbent assay (ELISA) technique for allergen-specific IgE determination, we observed an association among sensitization to cow's milk, beef and lamb in dogs diagnosed as suffering from CAFR. This association has also been observed in human allergy, but the molecular basis of

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this association has been studied only very recently. Thus, Ayuso *et al.*¹³ identified IgG (Bos d 7, according to the nomenclature of the International Union of Immunological Societies) as the major allergen responsible for allergy to beef in humans, and demonstrated a high degree of cross-reactivity with lamb and venison IgG. In addition, immunoglobulins are also present in milk and, consequently, they have an important role in cross-reactivity with meat. In another study, Fiocchi *et al.*¹⁴ identified bovine serum albumin (BSA, Bos d 6) as the major beef allergen in children, and found that beef-sensitive children were also sensitized to ovine serum albumin, as well as to other serum albumins. Like IgG, BSA is also a component of milk and may participate in cross-sensitization with meat. The other proteins with the strongest IgE-binding capacity identified in cow's milk are α -lactalbumin (Bos d 4), β -lactoglobulin (Bos d 5) and casein (Bos d 8).¹⁵

In this work, we aimed to identify the proteins present in cow's milk, beef and lamb that bind specific IgE in sera from dogs suffering from CAFR. As cow's milk proteins described as allergens in humans are commercially available, we first investigated the involvement of these allergens in canine CAFR to milk by means of a direct ELISA, and then studied the allergen composition of cow's milk, beef and lamb by SDS-PAGE followed by western blot.

MATERIALS AND METHODS

Extracts

To prepare lamb and beef extracts, meat was homogenized in a blender and stirred at a 10% (w/v) ratio in a NaCl solution (0.9%, w/v), for 2 h at 4 °C. The soluble fraction was separated by centrifugation at 12 000 g for 30 min at 4 °C, dialysed against distilled water, filtered through a 0.22 μ m filter and lyophilized. The cow's milk extract was prepared following essentially the same procedure from a 10% (v/v) dilution of milk in saline solution.

Protein concentrations of all extracts were determined using the method described by Bradford¹⁶ with BSA as standard.

BSA, IgG, α -lactalbumin, β -lactoglobulin and casein, and canine and ovine IgG were obtained from Sigma (St. Louis, MO, USA).

Sera

Sera were collected from 10 dogs with serum IgE specific to lamb, beef and cow's milk, as determined by PET® ELISA (Alergovet SL, Madrid, Spain). These dogs had a convincing history of CAFR to meat and cow's milk. Clinical manifestations included non-seasonal generalized pruritus with or without lesions. Commonly observed dermatological signs included papular or macular dermatitis (ears, rump, axillae, groin and distal limbs), superficial pyoderma, otitis externa and seborrhoea. Diagnosis of CAFR was confirmed by feeding an owner-prepared elimination diet for periods

ranging from 3 to 10 weeks. Protein sources included chicken meat, chicken eggs and salmon, and carbohydrate sources included rice and potatoes. The dietary period was concluded upon at least 50–75% resolution of pruritus. Dogs were then challenged with their regular diet, including cow's milk, beef and lamb. In all cases, exacerbation of pruritus was reported within 14 days of exposure to the original diet.

Sera from a group of 10 nonatopic dogs were included as negative controls in *in vitro* assays. Lamb, beef and cow's milk were ingredients of these dogs' regular diets.

To study the specificity of biotin-conjugated rabbit anti-canine IgE polyclonal antibody, sera from three dogs polysensitized to pollen and/or moulds were used.

Specificity of biotin-conjugated rabbit anti-canine IgE polyclonal antibody

Throughout this study, a biotin-conjugated rabbit anti-canine IgE polyclonal antibody (Alergovet SL) was used. This rabbit antiserum had been passed through an affinity column with dog IgG as immunosorbent until no reaction was detected in a direct ELISA with this immunoglobulin coupled to the solid phase (data not shown). The specificity of the biotin-conjugated anti-IgE antibody was assessed by a series of experiments as follows. First, recognition of thermolabile antigen-specific dog IgE antibodies was confirmed by heating serum samples from three polysensitized allergic dogs at 56 °C for 2 h¹⁷ and studying the reduction of binding after heating. Analysis was carried out by PET® ELISA with extracts of *Phleum pratense*, *Plantago lanceolata*, *Artemisia vulgaris*, *Ambrosia elatior* and *Cladosporium herbarum* on the solid phase. Results of this experiment are shown in Table 1. In all cases, a decrease in absorbance at 490 nm to values very close to background levels was observed. No significant changes were detected with the serum of a nonatopic dog used as a negative control. Further confirmation of the absence of cross-reaction of the rabbit anti-canine IgE antibodies with IgG from dog, cow or sheep was provided from the results obtained when negative controls using canine, bovine or ovine IgG, in the absence of dog sera, were included both in ELISA and western blots. These results are reported in the sections describing the results of the related experiments.

ELISA for allergen-specific serum IgE

As mentioned above, serum IgE against lamb, beef and cow's milk was determined using a commercial ELISA (PET®). The presence of specific IgE against purified proteins was detected using essentially the same method. Briefly, 96-well microtitre plates (NUNC Maxisorp plates, Roskilde, Denmark) were coated overnight at 4 °C with solutions of the pure proteins at an appropriate concentration (between 2 and 20 μ g/mL) in phosphate-buffered saline (PBS). After blocking with BSA, wells were sequentially incubated with samples (1:4 dilution), biotin-conjugated rabbit anti-canine IgE polyclonal antibody (1:5000 dilution) and streptavidin–peroxidase

Table 1. Binding of biotin-conjugated rabbit anti-canine IgE polyclonal antibody to thermolabile dog IgE antibodies

Allergosorbent	Serum							
	1		2		3		NC	
	T0	T120	T0	T120	T0	T120	T0	T120
<i>Phleum pratense</i>	0.814	0.113	1.909	0.123	1.258	0.082	0.057	0.042
<i>Plantago lanceolata</i>	0.430	0.106	1.662	0.131	0.100	0.062	0.041	0.047
<i>Artemisia vulgaris</i>	0.306	0.109	2.238	0.179	0.095	0.076	0.051	0.046
<i>Ambrosia elatior</i>	0.233	0.109	2.252	0.063	0.938	0.088	0.048	0.054
<i>Cladosporium herbarum</i>	2.053	0.071	0.101	0.066	0.032	0.030	0.041	0.024

Figures indicate absorbance at 490 nm in PET® ELISA with the indicated extracts adsorbed to the solid phase. Sera 1–3 were from polysensitized allergic dogs, and NC was from a nonatopic dog. Analyses were carried out on samples untreated (T0) or heated at 56 °C for 2 h (T120).

conjugate (1:1000 dilution, Vector Laboratories, Burlingame, CA, USA). Samples, controls and reagents were diluted in PBS containing 1% BSA and 0.1% Tween 20, and all incubations were carried out for 1 h at room temperature with intermediate washes between successive steps using PBS containing 0.1% Tween 20 (PBS-T). To determine specific IgE against BSA, this protein was replaced by casein in the dilution buffer. Finally, the wells were incubated in the dark for 15 min at room temperature with a solution of *o*-phenylenediamine (Sigma), and the colour reaction was stopped by adding 2 N HCl. The absorbance was read at 490 nm with a 650-nm reference filter using a microplate reader (Tecan, Durham, NC, USA).

SDS-PAGE and immunoblot analysis

SDS-PAGE was performed according to the method of Laemmli,¹⁸ at an acrylamide concentration of 15%. For reducing SDS-PAGE, 2-mercaptoethanol was added to the sample buffer up to a concentration of 5% (v/v). Commercial standard proteins were used as molecular mass markers (Novex, Carlsbad, CA, USA). Gels were stained with Coomassie Brilliant Blue to visualize protein bands or the separated proteins were electrotransferred onto a polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore, Bedford, MA, USA) according to the method of Towbin *et al.*¹⁹ PVDF sheets were either stained with Coomassie Brilliant Blue to detect the electrotransferred proteins or processed for immunoblot to identify IgE-binding proteins. In the latter case, the sheets were blocked in PBS-T for 30 min at room temperature, and then incubated with sera from allergic dogs (diluted 1:4 in PBS-T) for 2 h at room temperature. After washing with PBS-T, sheets were incubated with biotin-conjugated rabbit anti-canine IgE polyclonal antibody, diluted 1:10 000 in PBS-T, for 2 h at room temperature and washed again with PBS-T. After incubation with streptavidin–peroxidase conjugate (Jackson Immuno-research, West Grove, PA, USA) for 1 h at room temperature, sheets were washed with PBS-T and detection of IgE-binding proteins was accomplished by incubation with 3,3'-diaminobenzidine (DAB, Sigma). The reaction was stopped with deionized water and the blots were dried. Coomassie Brilliant Blue-stained gels and membranes, as well as immunoblots, were ana-

lysed using an image acquisition system and QUANTITY ONE software from Bio-Rad (Hercules, CA, USA).

N-terminal amino acid sequencing

N-terminal amino acid sequencing was carried out on electroblotted samples as described by Matsudaira *et al.*²⁰ using an Applied Biosystems Model 494 A liquid-pulse sequencer (Foster City, CA, USA).

Amino acid sequence comparisons were performed in protein and DNA sequence databases (GenBank, EMBL, PIR, NBR, NBR3D and Swiss Prot) using the FASTA program,²¹ version 3.3t09, at the ExPASy Molecular Biology Server of the Swiss Institute of Bioinformatics.

RESULTS

Identification of IgE-binding proteins in cow's milk

The presence of specific IgE against proteins of lamb, beef and cow's milk was studied in the individual sera of 10 dogs that had been diagnosed with CAFR to these foods. Table 2 shows that all the sera reacted to these extracts in an ELISA for specific IgE. In all cases, values for lamb extract were higher than those for beef and cow's milk, which gave similar results in most dogs. In contrast, individual sera from nonatopic dogs did not react with any of the extracts (data not shown). In Table 2, the results with a pool of these sera (NC) is included. Dog IgG was also included as allergosorbent in this experiment to assess the specificity of the biotin-conjugated anti-canine IgE (Table 2).

The serum IgE reactivity to pure proteins from cow's milk was also tested in the same assay (Table 2). Bovine IgG was recognized by all sera, whereas the other four proteins assayed did not react with any of the 10 sera. Furthermore, the absorbance values produced by the sera in the assay for IgG agreed well with those for cow's milk. These results demonstrate that bovine IgG is a major allergen responsible for canine CAFR to cow's milk. As bovine IgG can also be found in beef, it can be concluded that it is also involved in CAFR to beef. Likewise, all the sera showed reactivity to ovine IgG (Table 2), which suggests that IgG is a source of cross-reactivity with lamb and sheep's milk.

The results obtained by means of ELISA were corroborated by western blot. Figure 1 shows the results

Table 2. Specific IgE to lamb, beef and cow's milk extracts and to pure cow's milk proteins in serum samples from 10 allergic dogs

Allergosorbent	Serum No.										NC	Dil. Buffer
	1	2	3	4	5	6	7	8	9	10		
Lamb	2.068	2.768	1.069	2.636	1.151	2.033	0.987	2.375	1.996	2.620	0.091	0.074
Beef	0.676	1.082	0.433	1.333	0.552	0.889	0.393	0.916	0.639	1.063	0.083	0.072
Cow's milk	0.602	1.512	0.445	1.431	0.561	1.249	0.403	1.322	1.099	1.307	0.094	0.082
Bovine IgG	0.295	0.855	0.405	1.533	0.340	0.995	0.465	0.380	1.188	1.335	0.059	0.037
β -lactoglobulin	0.041	0.051	0.047	0.033	0.030	0.038	0.050	0.035	0.024	0.034	0.056	0.048
α -lactalbumin	0.074	0.042	0.052	0.050	0.049	0.037	0.042	0.043	0.040	0.045	0.062	0.054
BSA	0.059	0.062	0.050	0.048	0.073	0.008	0.041	0.037	0.057	0.025	0.059	0.060
Casein	0.052	0.042	0.053	0.042	0.052	0.030	0.051	0.024	0.045	0.023	0.075	0.059
Ovine IgG	0.267	0.611	0.335	1.313	0.275	0.697	0.428	0.331	0.974	1.135	0.062	0.049
Canine IgG	–	–	–	–	–	–	–	–	–	–	–	0.056

Figures indicate absorbance at 490 nm in an ELISA to detect specific IgE with the extracts or pure proteins adsorbed to microtitre plates, as described in Materials and Methods. Values represent the average from three experiments. NC, serum pool from 10 nonatopic dogs included as a negative control. The background of the assay with dilution buffer instead of serum sample is given in the last column. Canine IgG was included as a control of specificity of the rabbit anti-canine IgE polyclonal antibody used in the assay.

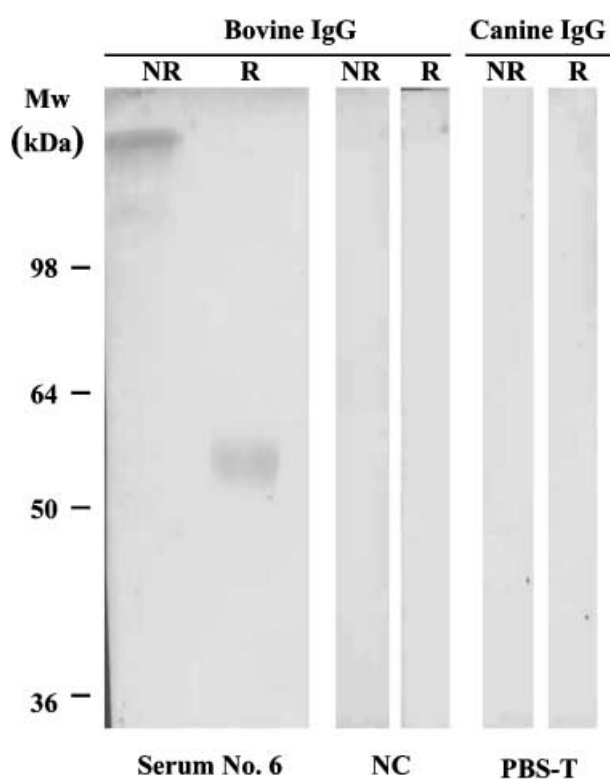


Figure 1. Immunodetection of bovine IgG after SDS-PAGE under nonreducing (lane NR) or reducing (lane R) conditions using serum no. 6 and a serum pool from nonatopic dogs. Canine IgG was processed in the same way and incubated with dilution buffer (PBS-T) instead of serum as a control of the specificity of the biotin-conjugated rabbit anti-canine IgE polyclonal antibody used. Molecular mass markers are indicated on the left.

of the immunodetection of bovine IgG using one of the sera (no. 6) with high levels of specific IgE against cow's milk and bovine IgG. Under nonreducing conditions (Fig. 1, lane NR), a sharp band with an apparent molecular mass of 160 kDa was detected, whereas under reducing conditions (Fig. 1, lane R) a broad band in the molecular mass range 53–58 kDa was immunostained, which correspond to the bovine IgG heavy chain. Neither the negative controls for bovine IgG immunodetection using sera from nonatopic dogs, nor

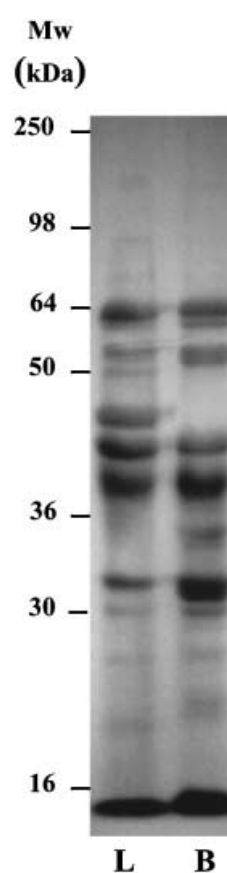


Figure 2. SDS-PAGE analysis of lamb (lane L) and beef (lane B) extracts under reducing conditions. Eighty micrograms of protein was applied per lane. Proteins were stained with Coomassie Brilliant Blue. Molecular mass markers are indicated on the left.

the control for specificity of biotin-conjugated rabbit anti-canine IgE using dog IgG on the membrane, produced any immunostaining (Fig. 1).

Identification of IgE-binding proteins in beef and lamb

Figure 2 shows the SDS-PAGE analysis of lamb and beef extracts. A similar pattern of bands was observed in both extracts, with only two remarkable differences,

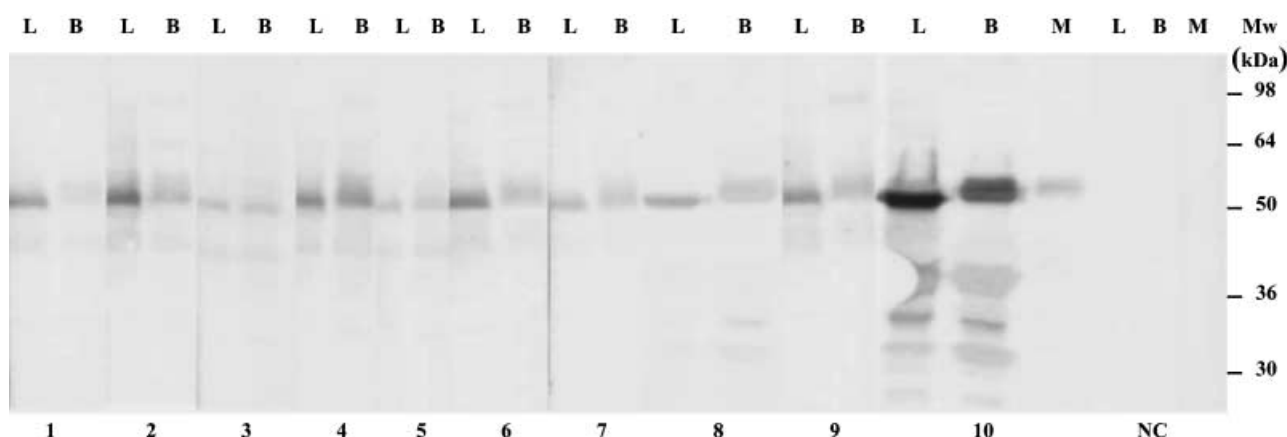


Figure 3. Immunodetection after SDS-PAGE of lamb (lanes L) and beef (lanes B) extracts with sera 1–10 and a serum pool from nonatopic dogs as a negative control (NC). Lanes M show the immunodetection of cow's milk extract using serum 10 and the negative serum pool. Electrophoresis was carried out under reducing conditions. Molecular mass markers are indicated on the right.

Figure 4. Comparison of the N-terminal amino acid sequence of the 51-kDa allergen identified in lamb extract with phosphoglucosutases of mammalian origin: human (Swiss Prot Accession no. P36871), rabbit (P00949), mouse (Q9D0F9) and rat (P38652). Asterisks indicate identity of amino acid residues in all the sequences. X = unidentified residue.

51-kDa Lamb allergen	VKIVTVKTXAYQ
Human phosphoglucosutase	VKIVTVKTQAYQ
Rabbit phosphoglucosutase	VKIVTVKTKAYP
Mouse phosphoglucosutase	VKIVTVKTQAYP
Rat phosphoglucosutase	VKIVTVKTQAYP
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the absence of a 45 kDa protein in beef extract and the different resolution of bands in the molecular mass range 50–60 kDa. In the lamb extract, a 51 kDa band was well resolved from a broad band corresponding to a molecular mass of 53–57 kDa, whereas in the beef extract these bands seem to be collapsed into a doublet.

The results of immunodetection of lamb and beef extracts with the individual sera are shown in Figure 3. The most strongly immunostained band of the lamb extract with all the sera corresponded to a protein with a molecular mass of 51 kDa (Fig. 3, lanes L). In the beef extract (Fig. 3, lanes B), two different patterns of reactivity were observed. Thus, sera 1, 6, 7 and 9 displayed a blurred band in the molecular mass range 53–58 kDa, similar to the band observed in the immunodetection of cow's milk extract (Fig. 3, serum 10, lane M), which suggests that it may correspond to the heavy chains of bovine immunoglobulins, either of the IgG class or not. The rest of the sera also showed the same 53–58 kDa diffuse band, but a more intense immunostaining was observed at the lower part of it, indicating that additional specific IgE is directed against the lower molecular mass protein in the doublet band of the SDS-PAGE pattern mentioned above, which could be homologous to the 51 kDa protein detected in lamb. This pattern is particularly clear in sera 3, 5, 8 and 10. Equally, most sera also produced a diffuse band above 51 kDa in the immunodetection of lamb extract, for example, sera 2, 4, 6 and 9. Other allergens, with similar molecular masses in both extracts, were visualized with some sera. Thus, immunoblots with sera 1–6 and 9 dis-

played a weak band at 42 kDa, and allergens with apparent molecular masses of 37, 33, 31 and 27 kDa were detected with serum 8 and, especially, serum 10.

The identity of the 51-kDa major lamb allergen was established by N-terminal amino acid sequencing of the first 12 residues on an electroblotted sample. The sequence obtained was compared with known protein sequences deposited in data banks. A significant sequence similarity was found with muscle phosphoglucosutase from different mammals (Fig. 4). Attempts to establish the N-terminal amino acid sequence of the homologue allergen in beef were unsuccessful. Multiple residues were identified in each sequencing cycle, probably due to the bad resolution of this protein from immunoglobulin heavy chains on SDS-PAGE.

Finally, an example of the results obtained when cow's milk extract was probed with dog sera in western blot is shown in Fig. 3, lane M. These results were similar to those obtained with bovine IgG (Fig. 1, lane R), corroborating that IgG is the major cow's milk IgE-binding protein.

DISCUSSION

Diagnosis of CAFR in pets is arduous because it relies on physical examination, clinical history and mainly on dietary investigation in the form of elimination diets until resolution of clinical signs followed by an appropriate exposure challenge. This methodology is labour-intensive, time-consuming and may place a great deal

of pressure on pet owners. This situation is not very different from diagnosis of food allergy in human patients, where the most conclusive diagnostic tool is the double-blind, placebo-controlled oral food challenge (DBPCFC),²² although the correlation of food allergies suspected by history with the results of DBPCFC found in some studies is notoriously poor.^{23,24} Nevertheless, recent advances in the purification, characterization and expression of a number of food allergens as recombinant proteins have allowed significant improvements of *in vitro* quantitative laboratory tests. Thus, Palosuo *et al.*²⁵ demonstrated that reactivity in a specific-IgE ELISA to a particular wheat protein, omega-5-gliadin, increased specificity for predicting IgE-mediated food allergy to wheat in children, and Jarvinen *et al.*²⁶ showed that the presence of IgE antibodies to distinct allergenic epitopes of cow's milk proteins can be used as a marker of persistent allergy to cow's milk. All these results indicate the relevance of characterizing food proteins at the molecular level for designing more specific and predictive *in vitro* tests. As a first step to improve *in vitro* diagnosis of IgE-mediated CAFR to cow's milk, beef and lamb in dogs, the objective of this study was the identification of the major allergens of these foods. We selected 10 dogs that had been diagnosed with CAFR by feeding an elimination diet and a subsequent exposure challenge, and that had serum-specific IgE to cow's milk, lamb and beef. In our study, all the dogs were positive to bovine IgG in a screening with pure cow's milk proteins, suggesting that the predictive value of the ELISA for serum-specific IgE using bovine IgG is at least as good as using cow's milk extract. Although these results should be confirmed by testing a larger number of patients comparing both allergosorbents, it seems obvious that when using a cow's milk extract in either an *in vitro* or *in vivo* test, the presence of a sufficient concentration of bovine IgG in the extract should be checked to avoid false-negative results.

In contrast to the findings reported in human allergy, we did not find canine serum IgE specific to other cow's milk proteins described as allergens.^{14,15} If these results are confirmed in further studies with larger populations of patients, it should be expected that development of epitope-based *in vitro* tests for cow's milk allergy in dogs could be straightforward.

Irrespective of other immunoglobulins that might also be involved in beef sensitization (a possibility that cannot be ruled out yet, as immunoglobulins of class distinct to IgG would give a similar pattern on SDS-PAGE), the results of ELISA experiments with bovine IgG and the fact that this class of immunoglobulin is present in beef indicates that it is a source of cross-reactivity between this meat and cow's milk. Moreover, it is a major beef allergen, because immunostaining of heavy chains of bovine immunoglobulins was observed with all the sera tested with a beef extract on SDS-PAGE immunoblot. Likewise, ovine IgG reacted with all the sera in ELISA and bands corresponding to heavy chains of immunoglobulins were detected in the

immunoblot of a lamb extract, suggesting that IgG is also a major lamb allergen, and it may play an important role in the cross-reactivity between bovine products and lamb. These results are in close agreement with those obtained by Ayuso *et al.*¹³ in human food allergy to beef, who found that bovine IgG is a major cross-reacting allergen with other mammalian meats, such as lamb and venison. In contrast, they reported a very weak cross-reactivity to pork and chicken. Therefore, these findings warrant further studies in order to investigate the possibility of including these meats as substitutes for beef and lamb in hypoallergenic diets for dogs.

Immunoblotting of the lamb extract with the individual sera revealed that all the dogs had specific IgE against a protein with a molecular mass of 51 kDa, whose N-terminal amino acid sequence is identical (except for one indeterminacy at position 9) to the human muscle phosphoglucumutase, and only differs in the amino acid residue at position 12 from the homologous proteins in rabbit, mouse and rat. Phosphoglucumutase is a ubiquitous enzyme, and is possibly present in all living systems. It is involved in the metabolism of glucose, catalysing the reversible conversion of glucose 1-phosphate and glucose 6-phosphate. Function/structure properties of phosphoglucumutases from mammalian tissues have been extensively characterized.^{27,28} The catalytically active enzyme is phosphorylated at Ser116 and requires a divalent metal ion as an activator for phosphate transfer steps. The crystal structure of rabbit muscle phosphoglucumutase has been determined, and the catalytic reaction centre and metal-binding domains have been identified.²⁸

To our knowledge, the allergenicity of lamb phosphoglucumutase has not been reported to date. Moreover, from the beef immunoblotting patterns, it can be suggested that phosphoglucumutase is also an important beef allergen, although the fact that this protein was not well resolved from heavy chains of bovine immunoglobulins on SDS-PAGE precluded its identification by N-terminal amino acid sequencing. As a matter of speculation, it is possible that beef phosphoglucumutase could be involved in human food allergy and its presence could have been overlooked because of the insufficient electrophoretic resolution from immunoglobulin heavy chains.

In general, the IgE-binding pattern to beef and lamb of sera from dogs included in this study was very similar. Thus, apart from the major allergens IgG and phosphoglucumutase, 7 of the 10 sera assayed reacted to a 42-kDa allergen and 2 others reacted to 37, 33, 31 and 27-kDa bands in both meats. Therefore, it seems that sensitization to a particular protein of bovine origin may elicit sensitization to the homologous ovine protein, and vice versa. Similar observations have been reported in human allergy.¹³

In conclusion, we identified the major allergens responsible for canine CAFR to cow's milk, beef and lamb. Our results provide evidence at the molecular level that lamb is not an appropriate meat for hypoallergenic

diets in dogs allergic to bovine products because sensitization to homologous proteins in both meats has been observed with all the sera assayed. The most important allergens seem to be IgG and phosphoglucomutase. As far as we know, this is the first time that phosphoglucomutase has been identified as a meat allergen. Our findings may help to pave the way for more specific and sensitive *in vitro* laboratory tests for IgE-mediated CAFR to major protein sources in diets of carnivore pets.

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Résumé La viande d'agneau, de boeuf, et le lait de vache sont des causes fréquentes d'intolérance alimentaire (CAFR) chez le chien. Le but de ce travail était d'identifier les protéines responsables de ces CAFR à ces aliments. Dix chiens présentant des IgE sériques à l'agneau, au boeuf ou au lait de vache ont été étudiés. Le diagnostic de CAFR a été réalisé chez ces animaux par la coexistence de signes cliniques compatibles, un régime d'éviction et des épreuves de provocation. Les séra ont été analysés par ELISA avec des protéines de boeuf, et par des immunoblots SDS-PAGE avec des extraits d'agneau, de boeuf et de lait de vache. Tous les chiens avaient des IgE spécifiques dirigées contre l'immunoglobuline G bovine, et il s'agissait de la seule protéine dans l'extrait de lait de vache qui liait les IgE dans les séra étudiés. Pour les extraits d'agneau et de boeuf, les allergènes majeurs reconnus par les IgE spécifiques dans les séra avaient un poids moléculaire de 51 à 58 kDa, et ont été identifiés comme une phosphoglucomutase et la chaîne lourde des IgG. D'autres protéines liant les IgE, d'un poids moléculaire de 27, 31, 33, 37 et 42 kDa ont également été identifiées dans certains séra. Nos résultats indiquent que l'IgG bovine est un allergène majeur dans le lait de vache et qu'elle est probablement une source de réaction croisée avec le boeuf, et probablement également avec l'agneau à cause de l'homologie importante qui existe entre les immunoglobulines ovines. Ces résultats sont semblables à ceux observés dans l'allergie aux viandes chez l'homme. Cependant, il s'agit de la première observation de l'identification de la phosphoglucomutase comme allergène responsable de réactions allergiques au boeuf et à l'agneau.

Resumen La carne de cordero, ternera y la leche de oveja son causas frecuentes de reacciones cutáneas alimentarias adversas en perros (CAFR). El objetivo de este trabajo fue identificar las proteínas responsables de las CAFR en estos alimentos. Diez perros con IgE de suero alérgeno-específico para cordero, ternera y leche de vaca fueron incluidos en el estudio. Estos perros habían sido diagnosticados con CAFR a través de una historia clínica convincente y pruebas de eliminación de alimentos seguidas por exposición tentativa. Los sueros fueron analizados mediante ELISA con proteínas bovinas e inmunoblots SDS-PAGE con extractos de cordero, ternera y leche de vaca. Todos los perros tenían IgE específicas contra inmunoglobulina G bovina, y fue la única proteína en el extracto de leche de vaca que se unió a IgE del suero estudiado. En los extractos de cordero y ternera, los alérgenos principales reconocidos por las IgE específicas de la mayoría de sueros tenían un peso molecular entre 51 y 58 kDa, que fueron identificados como fosfoglucomutasa y la cadena pesada de la inmunoglobulina G. También se detectaron en algunos sueros otras proteínas que se unían a IgE con pesos moleculares de 27, 31, 33, 37, y 42 kDa. Nuestros resultados indican que la IgG bovina es un alérgeno principal en la leche de vaca y por tanto parece ser una fuente de reactividad cruzada con ternera y probablemente con cordero debido a su elevada homología con las inmunoglobulinas ovinas. Estos resultados son similares a los obtenidos en la alergia a la carne en humanos. Sin embargo, ésta es la primera vez que la fosfoglucomutasa ha sido identificada como un alérgeno importante implicado en las reacciones alérgicas a cordero y ternera.

Zusammenfassung Lammfleisch, Rindfleisch und Kuhmilch sind häufige Auslöser von kutanen Nahrungsmittelunverträglichkeitsreaktionen (KNUR) bei Hunden. Ziel dieser Arbeit war es, die Proteine zu identifizieren, die für KNUR auf diese Futtermittel verantwortlich sind. Zehn Hunde mit allergen-spezifischen Serum-Ig-E auf Lammfleisch, Rindfleisch und Kuhmilch wurden in diese Studie eingeschlossen. Bei diesen Hunden wurde KNUR durch überzeugende klinische Vorgeschichte und Ausschlussdiät mit anschließendem Provokationstest diagnostiziert. Die Seren wurden anhand von ELISA mit bovinen Proteinen und SDS-PAGE-Immunoblot mit Lammfleisch-, Rindfleisch- und Kuhmilchextrakten analysiert. Alle Hunde hatten spezifisches Ig-E gegen bovines Ig-G und dies war das einzige Protein im Kuhmilchextrakt, das Ig-E in den untersuchten Seren gebunden hat. In den Lammfleisch-, Rindfleischextrakten hatten die vom spezifischen Ig-E der meisten Seren erkannten Major-Allergene ein Molekulargewicht zwischen 51 und 58 kDa, welche als Phosphoglucomutase und Schwerketten von Immunglobulin G identifiziert wurden. In einigen Seren wurden auch andere Ig-E bindende Proteine mit Molekulargewichten von 27, 31, 33, 37 und 42 kDa nachgewiesen. Unsere Ergebnisse weisen darauf hin, dass bovines Ig-G ein Major-Allergen in Kuhmilch ist und infolgedessen scheint es die Quelle für Kreuzreaktivität mit Rindfleisch und aufgrund der hohen Homologie mit ovinen Immunglobulinen vielleicht mit Lammfleisch zu sein. Die Ergebnisse sind denen ähnlich, die man bezüglich Fleischallergien beim Menschen gefunden hat. Es ist jedoch das erste Mal, dass Phosphoglucomutase als wichtiges Allergen bei allergischen Reaktionen auf Lamm- und Rindfleisch identifiziert wurde.