

ORIGINAL ARTICLE

ELISA testing for common food antigens in four dry dog foods used in dietary elimination trials*

D. M. Raditic, R. L. Remillard and K. C. Tater

MSPCA Angell Animal Medical Center, Boston, MA, USA

Keywords

elimination trial, food antigens, ELISA soy testing, ELISA beef testing, ELISA poultry testing, pet food contamination, dry food diets, canine

Correspondence

R. L. Remillard, MSPCA Angell Animal Medical Center, 350 S Huntington Ave, Boston, MA 02130, USA. Tel: 617 522 7282; Fax: 617 989 1670; E-mail: rremillard@mspca.org

*Study funded by MSPCA Angell Animal Medical Center, Resident Clinical Studies Grant, Boston, MA 02130, USA.

Received: 9 August 2009;
accepted: 8 February 2010

Summary

This study evaluated four over the counter venison dry dog foods available from one on-line retail vendor for potential contamination with common known food allergens: soy, poultry or beef. An amplified, double sandwich type enzyme linked immunosorbent assay (ELISA) test of soy, poultry and beef proteins were performed by an independent accredited food laboratory. The ELISA test for poultry protein was found to be unreliable when testing in dry dog foods because false negatives occurred. ELISA testing of control diets for both soy and beef proteins performed as expected and could be useful in antigen testing in dry dog foods. Three of the four over the counter (OTC) venison canine dry foods with no soy products named in the ingredient list were ELISA positive for soy; additionally one OTC diet tested positive for beef protein with no beef products listed as an ingredient list. One OTC venison diet was not found to be positive for soy, poultry or beef proteins. However, none of the four OTC venison diets could be considered suitable for a diagnostic elimination trial as they all contained common pet food proteins, some of which were readily identifiable on the label and some that were only detected by ELISA. Therefore, if the four OTC venison products selected in this study are representative of OTC products in general, then the use of OTC venison dry dog foods should not be used during elimination trials in suspected food allergy patients.

Introduction

The primary symptoms of food allergies are a non-seasonal pruritus but also include bilateral otitis, bacterial pyoderma and secondary seborrheic skin disease. Concurrent gastrointestinal signs are common. Currently, the definitive diagnosis of food allergy in dogs is based on results of an elimination trial followed by a food challenge test. After a successful dietary elimination trial with resolution of skin and/or gastrointestinal signs a food challenge is performed. The dog is fed the previous diet, which should elicit the original symptoms. Refeeding the successful food trial diet should resolve the signs if the dog has a true food allergy (Scott et al., 2001).

Oral tolerance is both locally and systemically an immunologic 'unresponsiveness' to an antigen presented in the gut. This suppressor function is regulated by gut associated lymphoid tissue. The mucosal immune system encounters enormous quantities of antigens daily and generally suppresses immune reactivity to many harmless food antigens. In food allergies it has been proposed oral tolerance fails to develop normally or breaks down (Verlinden et al., 2006).

It is hypothesized that immunological food reactions develop when a food specific IgE antibody on a mast cell binds with a food antigen. This binding initiates mast cell release of potent inflammatory mediators and cytokines (Sampson, 2004). It is believed

that most food allergies are Type I or IgE mediated but there is some evidence to suggest Type III and IV hypersensitivity reactions may be involved (Verlinden et al., 2006).

This immunologic response to a food borne antigen must be a protein or a glycoprotein in order to initiate an antigen/antibody complex and the resulting immune cascade that causes inflammation in the skin or gastrointestinal tract (Roudebush et al., 2000). The meat proteins commonly used in pet foods are beef, poultry, egg, fish and pork. The most common grain proteins are soybean, rice, corn and wheat (Cowell et al., 2000). Dogs have been reported to have food allergies to beef, dairy products, wheat, lamb, egg, chicken and soy (Roudebush et al., 2000). Food allergies to rice have been reported (Scott et al., 2001).

When a pet develops a food allergy it is most often after chronic exposure to one of these common dietary proteins. Hence, diets used in diagnostic elimination trials must contain novel protein sources, i.e. food proteins never before consumed by the patient. The diet also cannot contain any amount of the more commonly used protein ingredients. Known and unknown consumption of common allergen proteins during the food trial nullify or confound the results of such a trial and preclude an accurate diagnosis. (Roudebush et al., 2000). Intentional or known consumption of a suspected allergen occurs when the owner gives an unapproved treat during a food trial; whereas an unknown or unintentional consumption of an allergen would occur when a dog food contains ingredient residues from other products, i.e. ingredient cross contamination during manufacturing.

General practitioners, gastroenterologists and dermatologists use dietary elimination trials to diagnose food allergies in dogs. These trials are lengthy (4–13 weeks) and require a client's strict adherence to the prescribed food. There has been an increase in pet owner interest in using over the counter (OTC) commercial diets sold through retail outlets for a diagnostic dietary elimination trial because of price and convenience (Scott et al., 2001). General practitioners and some veterinary specialists have acquiesced to such client requests, not fully understanding the difference and value in product quality control between veterinary therapeutic and OTC commercial lines of pet foods. The pet owner may select OTC diets based on the name of the product, e.g. venison and sweet potato, which ensures nothing more than the product will contain at least 3% of those ingredients (Cowell et al.,

2000). Pet owners and veterinarians also make the assumption that if food proteins or isolates of a food protein are not named in the product ingredient list, then the product does not contain those food proteins and therefore, is a suitable diet to be used in a diagnostic elimination trial (Scott et al., 2001). Our hypothesis was that OTC commercial novel or exotic protein (e.g. venison) diets do not contain common food proteins specifically soy, poultry or beef unless specified on the label.

Materials and methods

Four OTC canine dry food diets available through one on-line retail vendor during June 2009 with the novel source of protein, venison, in the product name were selected (Table 1) as test diets. Venison or venison meal had to be listed as the first or second ingredient and the list could not contain a soy or beef ingredient. The presence of poultry in the ingredient list was avoided if possible but allowed because it was common. A negative control canine dry venison diet (Table 1) was selected based on these criteria except availability was only through a veterinarian. The positive control canine dry OTC diets (Table 1) available through a local retail vendor at the same time were selected based on the ingredient list specifically containing a soy, poultry and/or beef product, regardless of the product name. All diets were appropriate for feeding adult maintenance dogs and sold as complete and balanced according to AAFCO regulations.

All control diets were tested for the presence of soy, beef and poultry to test for cross reactivity (e.g. beef vs. venison vs. poultry; meat (clean flesh) vs. by-product). There was no comparable test available specifically for venison. One 100 g sample of each diet was sent to an independent laboratory (Elisa Technologies, Gainesville, FL 32653; <http://www.elisa-tek.com>. Certificate Number: 2793.01) using overnight delivery to test for the presence of soy, poultry and beef antigens. The laboratory was blinded to the product name and ingredient list of the diets. The laboratory was accredited (ISO 17025; International Organization for Standardization. http://www.iso.org/iso/catalogue_detail.htm?csnumber=39883.) in meat speciation and allergen testing. The laboratory routinely performed enzyme linked immunosorbent assay (ELISA) testing in quadruplicate for the presence of antigens in food products, feed, drugs, food additives and unknown biological samples.

Table 1 Control and test canine dry dog food product ingredient lists

Diets	Purpose	Ingredient list as appearing on the product ^a
Control		
1	negative control ^b	Dehydrated potato, Venison meal, Potato protein, Coprah oil, Natural flavors, Venison, Anchovy oil, Soya oil, Salt, L-tyrosine, dl-methionine, Taurine, Vitamins (dl-alpha tocopherol acetate (source of vitamin E), Inositol, Niacin supplement, l-ascorbyl-2-polyphosphate (source of vitamin C), d-calcium pantothenate, biotin, pyridoxine hydrochloride (vitamin B6), Riboflavin supplement (vitamin B2), Thiamine mononitrate (vitamin B1), Vitamin A acetate, Folic acid, Vitamin B12 supplement, Vitamin D3 supplement, Choline chloride, Trace Minerals (Zinc proteinate, Zinc oxide, Ferrous sulfate, Manganese proteinate, Copper proteinate, Copper sulfate, Manganous oxide, Calcium iodate, Sodium selenite), Rosemary extract, Preserved with natural mixed tocopherols (source of vitamin E) and Citric acid
2	positive control for soy protein ^c	Whole grain corn, Poultry meal, Soybean meal , Beef fat, Brown rice, Apples, Peas, Carrots, Animal digest, Calcium carbonate, Dicalcium phosphate, Salt, Choline chloride, Potassium chloride, Vitamins (Vitamin E supplement, Vitamin A supplement, Niacin supplement, D-calcium pantothenate, Riboflavin supplement, Pyridoxine hydrochloride, Thiamine mononitrate, Vitamin D3 supplement, Folic acid, Biotin, Vitamin B12 supplement), Minerals (Ferrous sulfate, Zinc oxide, Manganese oxide, Copper sulfate, Calcium iodate, Sodium selenite), Caramel coloring, Yellow 5, Red 40, Blue 1, Blue 2, Rosemary extract
3	positive control for poultry striated meat ^d	Chicken , Ground whole grain corn, Ground whole grain sorghum, Ground whole grain wheat, Chicken by-product meal, Soybean meal , Corn gluten meal, Animal Fat, Brewers rice, Chicken liver flavor, Soybean Oil, Flaxseed, Potassium chloride, Iodized salt, Calcium carbonate, Choline chloride, Vitamin E supplement, Vitamins (l-ascorbyl-2-polyphosphate, Vitamin E supplement, Niacin, Thiamine mononitrate, Vitamin A supplement, Calcium pantothenate, Biotin, Vitamin B12 supplement, Pyridoxine hydrochloride, Riboflavin, Folic Acid, Vitamin D3 supplement), Minerals (Ferrous sulfate, Zinc oxide, Manganous oxide, Copper sulfate, Calcium iodate, Sodium selenite), dl-Methionine, Beta-carotene, Rosemary extract
4	positive control for bovine striated meat ^e	Ground corn, Chicken-by-product meal , Corn gluten meal, Animal fat, Beef , Whole grain brown rice, Rice, Natural poultry flavor, Dried peas, Wheat flour, Dicalcium phosphate, Potassium chloride, Salt, Vegetable oil, Carmel color, Dried beet pulp, Vitamins (dl-alpha tocopherols acetate, Choline chloride, l-ascorbyl-2-polyphosphate, Vitamin A acetate, Thiamine mononitrate, d-calcium pantothenate, Biotin, Riboflavin supplement, Vitamin D3 supplement, Vitamin B12 supplement), Calcium carbonate, Dried vegetables (carrots, spinach, tomato), Taurine, Titanium dioxide, Iron oxide, Minerals (Zinc sulfate, Zinc proteinate, Copper sulfate, Copper proteinate, Manganese proteinate, Potassium iodide), Mixed tocopherols, Marigold extract, Chlorophyll
Test		
5	OTC venison and potato diet ^f	Venison, Potato, Ground whole grain sorghum, Brewer's rice, Ground whole grain barley, Chicken meal , Fish meal, Chicken fat, Dried egg product, Dried beet pulp, Potassium chloride, Chicken flavor, Brewer's dried yeast, Fish oil, Salt, Vitamins (Vitamin E, Ascorbic acid, Beta carotenes, Vitamin A acetate, Calcium pantothenate, Biotin, Vitamin B12 supplement, Thiamine mononitrate, Niacin, Riboflavin supplement, Inositol, Pyridoxine hydrochloride, Vitamin D3 supplement, Folic acid), Sodium hexametaphosphate, Choline chloride, Minerals (Ferrous sulfate, Zinc oxide, Manganese sulfate, Copper sulfate, Manganous oxide, Potassium iodide, Cobalt), dl-methionine, Calcium carbonate, Rosemary extract
6	OTC sweet potato and venison ^g	Sweet potato, Venison, Potato protein, Venison meal, Canola oil, Potato fiber, Flaxseed, Natural flavor, Choline chloride, Natural mixed tocopherols, Taurine, Vitamin E, Iron proteinate, Zinc proteinate, Copper proteinate, Ferrous sulfate, Zinc sulfate, Copper sulfate, Potassium Iodide, Thiamine mononitrate, Manganese proteinate, Manganous oxide, Ascorbic acid, Vitamin A supplement, Biotin, Calcium pantothenate, Manganese sulfate, Sodium selenite, Pyridoxine hydrochloride, Vitamin B12 supplement, Riboflavin, Vitamin D3 supplement, Folic acid
7	OTC venison meal and millet ^h	Venison meal, Millet, Oatmeal, Barley, Canola oil, Pumpkinseeds, Montmorillonite clay, Natural lamb flavor, Potassium chloride, Vitamins (Choline chloride, Vitamin E supplement, Ascorbic acid, Biotin, Niacin, Vitamin A acetate, d-calcium pantothenate, Riboflavin, Pyridoxine hydrochloride, Ethylenediamine dihydriodide, Thiamine, Vitamin B12 supplement, Beta-carotene, Vitamin D3 supplement, Folic acid), Sea salt, Minerals (Zinc proteinate, Iron proteinate, Manganese proteinate, Copper proteinate, Sodium selenite), Chicken livers , Inulin, Amaranth, Quinoa, Blueberries, Cranberries, Dried <i>Enterococcus faecium</i> , Dried <i>Lactobacillus acidophilus</i> , Freeze dried venison, Pineapple, Rosemary extract, Sage, Freeze dried lamb hearts, Freeze dried lamb liver, Apples, Carrots, Ground lamb bone, Butternut squash, Ground flaxseeds, Chicken eggs, Broccoli, Lettuce, Spinach, Dried kelp, Salmon oil, Apple cider vinegar, Parsley, Honey, Alfalfa sprouts, Persimmons, Olive oil, Duck eggs, Pheasant eggs, Quail eggs, Sage, Clove

Table 1 (Continued)

Diets	Purpose	Ingredient list as appearing on the product ^a
8	OTC rice and venison ⁱ	Ground rice , Deboned venison, Rice protein concentrate, Sunflower oil, Flaxseed, Dicalcium phosphate, Natural venison flavor, Calcium carbonate, Potassium chloride, Vitamins (Beta-carotene, Vitamin E supplement, Ascorbic acid, Vitamin A supplement, Niacin, Calcium pantothenate, Riboflavin, Vitamin D3, Pyridoxine hydrochloride, Thiamine mononitrate, Folic acid, Biotin, Vitamin B12 supplement), Minerals (Zinc proteinate, Zinc sulfate, Iron proteinate, Ferrous sulfate, Copper sulfate, Copper proteinate, Manganese proteinate, Manganese sulfate, Calcium iodate, Sodium selenite, Choline chloride), Taurine

OTC, over the counter.

^aBolded ingredients are discussed in the text.

^bRoyal Canin Veterinary Diet Canine Venison and Potato Formula, Royal Canin, St. Charles, MO, USA.

^cWholesome Medley, Del Monte Pet Products, Pittsburgh, PA, USA.

^dScience Diet Adult Advanced Fitness, Hill's Pet Products, Topeka, KS, USA.

^eGood Life Recipe, The Good Recipe Petcare Company. A Division of Mars, Franklin, TN, USA.

^fEukanuba Naturally Wild New Zealand Venison & Potato, The Iams Company. Columbus, OH, USA.

^gDick Van Patten's Natural Balance Pet Foods, L.I.D., Limit Ingredient Diet Sweet Potato & Venison, Dick Van Patten's Natural Balance Pet Foods, Pacoima, CA, USA.

^hNature's Variety Prairie New Zealand Venison Meal & Millet Medley, Nature's Variety. Lincoln, NE, USA.

ⁱWellness Simple Food Solutions Rice & Venison, Old Mother Hubbard. Chelmsford, MA, USA.

Soy test

A USDA approved amplified, double sandwich type ELISA utilizing specific anti-soy trypsin inhibitor and other soy protein antibodies was used for soy antigen testing on all eight diets. The methodology of this test is based on the technique as described by Brandon et al., 1988, 1989. Internal positive and negative controls conducted simultaneously with test samples validate the kit. For the test to be valid, the controls correspond to the kit performance criteria as set out by the Certificate of Analysis for each specific lot number. The ELISA test is positive for soy protein and quantitative when compared with a known soy flour protein concentration gradient between 2.5 and 25 ppm. The lower limit of detection for this assay is the value of the lowest positive control which is 2.5 ppm soy flour protein. A sample with less than 2.5 ppm soy should not be interpreted as having no soy but simply as containing less than 2.5 ppm. The level of soy protein in a test sample will vary according to ingredients and manufacturing. Soy protein that has been significantly treated or altered with high temperatures, pressure, fermentation or hydrolysis may not be detected.

Beef and poultry test

An amplified, double sandwich type ELISA utilizing a biotinylated beef and poultry antibody was used on all the eight diets. This cooked meat species

ELISA was developed and is utilized as described in the USDA Laboratory Guidebook Notice of Change (2005). The assay is based on the antibodies raised to heat-resistant, species specific muscle related glycoproteins. The tests are sensitive and specifically designed to detect cooked meat species in cooked meat, meat products, meat and bone meals and heat processed foods. Kit validation is met when the mean absorbance value of the positive controls is greater than 0.600, the standard deviation of the replicates must not be more than 0.600 and the mean of the negative control must be less than 0.060. Test samples with a mean absorbance value minus three standard deviations greater than 0.250 are considered positive; all other samples are negative.

As described in the USDA Laboratory Guidebook Notice of Change (2005) specificity is based on species specific reagents tested against a panel of meat samples for cross reaction and has been found to produce negative responses to heterologous species samples. The poultry ELISA was positive for striated muscle of all feathered species including chicken, turkey, game hens, goose and duck. The cooked beef test was positive for both beef and bison striated muscle, as these are closely related species. The lower limit of detection for poultry was 4% and 1% for beef in a mixed extract of the target and a non-target species. There are limitations to the quantification of muscle in a sample because muscle antigens are heat labile. Temperature and cooking times of a pet food product can result in lower antibody/

antigen reaction for beef and poultry. Therefore, the intensity of the ELISA reaction is related to the extent of sample processing as well as to the level of antigen present (Laboratory Guidebook Notice of Change, 2005).

Source of protein antigens in the ingredient 'beef' would be the flesh derived from slaughtered bovine limited to striated muscle or that found in the tongue, diaphragm, heart or esophagus with and without normally accompanying fat, skin, sinew, nerve and blood vessels. Source of protein antigens in the ingredients 'chicken' and 'chicken or poultry meal' would be the flesh derived from slaughtered poultry exclusive of feathers, heads, feet and entrails. Source of protein antigens in the ingredient 'chicken by-product meal' would be the parts of the slaughtered poultry carcass such as necks, feet, undeveloped eggs and intestines, exclusive of feathers (AAFCO, 2009a).

Results

Control diets

The ELISA results are presented in Table 2. The negative control veterinary therapeutic diet (diet 1) was found to contain less than 2.5 ppm soy and negative for beef and poultry in ELISA tests. The test results of this product were consistent with the product label and venison, assuming it was present in the diet, did not cross react with beef antibodies. The positive over the counter control diets (diets 2–4) were, respectively, positive for soy, poultry and beef

protein as expected according to label ingredients. Hence, the control diets, used to detect a specific protein, confirmed the use of these ELISA tests to detect the presence of soy, poultry and beef protein in canine dry foods. Additionally, control diet 4 was also positive for soy but did not list soy in the ingredient list. Conversely, control diet 2 listed poultry meal and diets 4 listed chicken by-product meal in the ingredients listed but did not test positive for poultry.

Test diets

The OTC venison canine dry food test diets 5–7 did test positive for soy while test diet 8 contained less than 2.5 ppm soy. Diet 6 was positive for beef with no beef listed on the label. Test diet 5 contained chicken meal and tested positive for poultry while test diet 7 contained chicken livers but was negative on the poultry test. Test diet 8 was not found to be positive for beef or poultry protein.

Discussion

The OTC venison diets (5–7) did contain common food proteins, not found in the ingredient list which, makes them unsuitable for elimination trials. Proteins soy, poultry or beef were not detected in OTC venison diet 8 but the carbohydrate source rice is a common grain source found in many dry dog foods. Many dogs in the USA would have a history of rice protein exposure; therefore, diet 8, may not be a suitable diet for elimination trials.

The discrepancies between the ELISA results and the product labels for OTC diets (4–7) is key to understanding why OTC diets using novel or exotic names or ingredients may not be suitable in a diagnostic food elimination trial. OTC diets 4–7 tested positive for soy protein with no identifiable source of soy listed on the labels. In fact, diet 7 claimed on the display panel of the product that it contained 'no soy'. The ELISA testing of foods is used to detect soy contamination for soy allergic people. Soy protein concentrations low as 10 ppm may invoke a reaction in a soy allergic person (Koppelman et al., 2004). The lower limit of detection in dogs is not known. Immunoassay kits developed to detect the presence of soy protein in various foods are very specific and do not detect trypsin inhibitors from other legumes such as lima beans or chickpeas (Brandon and Friedman, 2002). Based on the possibility of soy protein contamination in OTC dry dog foods and if the four OTC diets selected in this study are

Table 2 ELISA results of control and test canine dry dog foods

Diets	Tested ingredient named in the ingredient list	ELISA			Test results match ingredient list?
		Soybean ppm	Beef	Poultry	
Control					
1	None	<2.5	neg	neg	yes
2	Poultry meal, Soybean meal	>25	neg	neg	no
3	Chicken, Chicken by-product meal, Soybean meal	>25	neg	pos	yes
4	Chicken by-product meal, Beef	21.8	pos	neg	no
Test					
5	Chicken meal	8.5	neg	pos	no
6	None	>25	pos	neg	no
7	Chicken livers	22.0	neg	neg	no
8	None	<2.5	neg	neg	yes

representative of similar products then OTC dry dog foods should not be used during elimination trials in suspected soy allergic patients.

The ELISA test for poultry did not provide consistent results. Although the test was positive for the control diet 3 which listed chicken and chicken by-product meal on the label, the test was not test positive for poultry meal, chicken by-product meal or chicken liver ingredients listed in diets 2, 4 and 7 respectively. The poultry ingredients in diets 2, 4 and 7 were not detected most likely due to the low concentration of striated muscle. The concentrations of antigen would be affected by processing e.g. temperatures or cooking times; a known limitation of the cooked meat ELISA tests. This test would not be expected to be positive for eggs in diet 7. The antigen in egg, α -lividen, is not a striated muscle protein (Cahen et al., 1998). Based on the results in this small sample of dog foods, the poultry cooked meat ELISA test should not be considered a reliable test for poultry antigen detection in dry dog foods due to false negatives.

The beef meat ELISA test did test positive in the control diet 4 and negative in all other control diets as expected. OTC diet 6 tested positive for beef with no beef in the ingredient list. Given beef did not cross react with venison; it is likely that OTC diet 6 was contaminated with beef muscle proteins. This was considered important as beef may be the most prevalent food antigen in dogs (White 1998), whereas in humans, objective measurements of food allergens suggests soy and wheat to be the most common plant allergens (Zuidmeer et al., 2008). Given no false negatives were detected in this limited study, the cooked beef ELISA test maybe useful when testing for beef antigen in dry dog foods. This test requires more stringent evaluation in dry dog foods.

The difference between the soy and beef protein ELISA test results and product labelling raises concerns about both labelling and manufacturing techniques. OTC diets that contain common food proteins but do not list these proteins on the product label may occur for several reasons. Pet food labels should report all ingredients used in the making of the product with few exceptions. One known exception is the addition of certain ingredients that are used to facilitate the manufacturing process. These special purpose products include verxite, ball clay, diatomaceous earth, disodium EDTA, hydrophilic silica, mineral oil and others as listed in AAFCO, 2009b. These are anti-caking agents, flow ingredients, dust control agents and lubricants used to affect stability, form or other technical qualities of the food

(National Research Council of the National Academies, 2006). It seems unlikely these products would be detected by either the soy or beef ELISA because most are inorganic compounds, oils, minerals or specific plant by products, which contain no soy, poultry or beef proteins. These ingredients typically comprise <0.1% of the diet and are destroyed by processing (Cowell et al., 2000).

The presence of soy and beef protein in these dry dog food diets is most likely due to ingredient cross contamination. This contamination may occur during manufacturing in a wide variety of ways. Grain and meat meal ingredients are purchased in large quantities and transported to the pet food manufacturing plant by truck (25 tons/load) or rail (90 tons/railcar) (Cowell et al., 2000). Bulk quantities of different ingredients are ground or dried using the same high volume equipment. Cross contamination can occur when transportation vehicles and/or equipment used for different ingredients at different times are not thoroughly cleaned in between the different ingredient types. Bulk ingredients are also blown into mixing hoppers using forced air and air borne particles may cross contaminate other nearby ingredients. Freeze dried meal ingredients are held as large unprotected piles in warehouses open to contamination by other ingredient particles. Cross contamination between different ingredients is allowable within reason; 'practices should minimize the potential for contamination' and contamination may occur 'as unavoidable under good processing practices' or 'except in such amounts as might occur unavoidably in good processing practices' according to AAFCO, 2009c.

Co-manufacturers or co-packers produce pet food products for both small and large companies as well as private label companies and may be the common source of a contaminate as exemplified by the March 2007 pet food recall (Burns, 2007). Small companies typically rely on a co-manufacturer for the entire process from product formulation, ingredient supply, packaging and quality control. A large company may use a co-packer when a special piece of equipment is required to make a particular product (e.g. gravy in pouches) or when additional capacity is needed. Large companies that use co-packers generally use their own ingredients, food scientist, engineers and quality control processes (Kirk, 2007). It is possible within the labelling regulations for co-packers or suppliers to substitute or add ingredients (Burns, 2007). Co-packing companies have the advantage of large capacity and specialization which lowers production cost for an individual pet food companies

but increases the possibility of product contamination.

Knowing cross contamination is possible in the manufacturing process, each pet food company should have quality control procedures in place to monitor product consistency. Product quality control cannot be ascertained by examining the product label (Kirk, 2007). Large companies usually have very complex and detailed quality control procedures with continuous monitoring. Small and specialty food companies may not have the same level of quality control and continuous oversight due to expense. According to Kirk (2007), the quality control is generally considered excellent in the global pet food companies but varies from excellent to non-existent in smaller companies. It is not clear what factors would be taken into consideration in determining a large vs. small company or what is good vs. poor quality control and such distinctions are beyond the scope of this study. Contamination with soy or beef protein was found in products (diets 4–7) made by several different size pet food companies in this study.

Pet food manufacturers generally use standards of quality control appropriate for the intended use of their product (Kirk, 2007). The OTC venison dry dog foods tested in this study were not intended by their respective manufacturers to be used in diagnostic elimination trials. The companies making veterinary therapeutic diets designed specifically to be used in elimination trials use highly sophisticated levels of ingredient and product quality control measures to prevent food antigen contamination from the first handling of raw ingredients to the final stages of packaging (Kirk, 2007).

In conclusion, three of the four OTC venison diets selected in this study contained at least one common food antigens, as detected by ELISA testing and are not suitable for diagnostic elimination trials. It is not known what protein concentration, size or structure is required to elicit an immunological reaction in a food allergic dog (Jenkins et al., 2007). Although, the exact mechanism of food allergies in dogs is still speculative, the immunological basis dictates the necessity to use diets with novel or hydrolyzed sources of proteins from companies with the highest quality controls, monitoring and assurances possible when conducting a diagnostic elimination trial.

References

- AAFCO, 2009a: Official names and definitions of feed ingredients as established by the Association of American Feed Control Officials. In: Official Publication of the Association of American Feed Control Officials. 414–425.
- AAFCO, 2009b: Feed terms and ingredient definitions. In: Official Publication of the Association of American Feed Control Officials. 411–421.
- AAFCO, 2009c: Guidance/Framework for best management practices for manufacturing, packaging and distributing animal feeds and feed ingredients. In: Official Publication of the Association of American Feed Control Officials. 239–244.
- Brandon, D. L.; Friedman, M., 2002: Immunoassays of soy protein. *Journal of Agricultural and Food Chemistry* **50**, 6635–6642.
- Brandon, D. L.; Bates, A. H.; Friedman, M., 1988: Enzyme-linked immunoassay of soybean Kunitz trypsin inhibitor using monoclonal antibodies. *Journal of Food Science* **53**, 102–106.
- Brandon, D. L.; Bates, A. H.; Friedman, M., 1989: Monoclonal antibody-based enzyme immunoassay of Bowman–Birk protease inhibitor of soybeans. *Journal of Agricultural and Food Chemistry* **37**, 1192–1196.
- Burns, K., 2007: Events leading to the major recall of pet foods. *Journal of the American Veterinary Medical Association* **23**, 1600–1620.
- Cahen, Y. D.; Frisch, R.; Wuthrich, B., 1998: Food allergy with monovalent sensitivity to poultry meat. *Clinical and Experimental Allergy* **28**, 1026–1030.
- Cowell, C. S.; Stout, N. P.; Brinkmann, M. F.; Moser, E. A.; Crane, S. W., 2000: Making of commercial pet foods. In: M. S. Hand, C. D. Thatcher, R. L. Remillard, P. Roudebush (eds), *Small Animal Clinical Nutrition*, 4th edn. Walsworth Publishing Company, Marceline, USA, pp. 127–146.
- Jenkins, J.; Brieteneder, H.; Millis, N. C., 2007: Evolutionary distance from human homologs reflects allergenicity of animal food proteins. *Journal of Clinical Immunology* **120**, 1399–1405.
- Kirk, C.: 2007. Overview of pet food manufacturing. In: *Current Issues in Small Animal Nutrition*. Veterinary Information Network: <http://www.vin.com/Members/CMS/Rounds>. Davis, CA, USA.
- Koppelman, S. J.; Lakemond, C. M.; Wlooswijk, R.; Hefle, S. L., 2004: Detection of soy protein in processed foods: literature overview and new experimental work. *Journal of AOAC International* **87**, 1398–1403.
- Laboratory Guidebook Notice of Change, 2005: In: United States Department of Agriculture, Laboratory QA/QC Division. Section 17.1; 950 College Station Road; Athens, GA 30605, USA. 2–13.
- National Research Council of the National Academies, 2006: Diet formulations and feed processing. *Nutrient Requirements of Dogs and Cats*. The National Academies Press, Washington, DC, USA, pp. 313–318.

- Roudebush, P.; Guilford, W. G.; Shanley, K. J., 2000: Adverse reactions to food. In: M. S. Hand, C. D. Thatcher, R. L. Remillard, P. Roudebush (eds), *Small Animal Clinical Nutrition*, 4th edn. Walsworth Publishing Company, Marceline, USA, pp. 432–446.
- Sampson, H. A., 2004: Update on food allergy. *Journal of Allergy Clinical Immunology* **113**, 805–819.
- Scott, D. W.; Miller, W. H.; Griffin, C. E. eds. 2001: Canine food hypersensitivity. In: *Muller and Kirk's Small Animal Dermatology*, 6th edn. WB Saunders Company, Philadelphia, PA, USA, pp. 615–627.
- Verlinden, A.; Hesta, M.; Millet, S.; Janssens, G. P., 2006: Food allergy in dogs and cats: a review. *Clinical Reviews in Food Science and Nutrition*. **46**, 259–273.
- White, S. D., 1998: Food allergy in dogs. *The Compendium for Small Animal Practitioners*. **20**, 261–268.
- Zuidmeer, K. G.; Goldhahn, K.; Rona, R. R.; Gislason, D.; Madsen, C.; Summers, C.; Sodergren, E.; Sahistrom, J.; Lindner, T.; Sigurdardottir, S.; McBride, D.; Keil, T., 2008: The prevalence of plant food allergies: a systemic review. *Journal of Allergy Clinical Immunology* **121**, 1210–1218.