4-2015

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Tara A. Okuma
Chapman University

Rosalee S. Hellberg
Chapman University, hellberg@chapman.edu

Recommended Citation
Okuma TA, Hellberg RS. 2015. Identification of meat species in pet foods using a real-time polymerase chain reaction (PCR) assay. Food Control 50: 9-17.
doi: 10.1016/j.foodcont.2014.08.017

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Identification of Meat Species in Pet Foods Using a Real-time Polymerase Chain Reaction (PCR) Assay

Authors: Tara A. Okuma[a], Rosalee S. Hellberg[b*]

[a]Chapman University, Schmid College of Science and Technology, Biochemistry and Molecular Biology, One University Drive, Orange, CA USA 92866

[b]Chapman University, Schmid College of Science and Technology, Food Science and Nutrition, One University Drive, Orange, CA USA 92866

[*]Corresponding author: Rosalee S. Hellberg, e-mail: hellberg@chapman.edu, Ph: +1-714-628-2811

Abstract

Product mislabeling, adulteration, and substitution are increasing concerns in highly processed foods, including pet foods. Although regulations exist for pet foods, there is currently a lack of information on the prevalence of pet food mislabeling. The objective of this study was to perform a market survey of pet foods and pet treats marketed for domestic canines and felines to identify meat species present as well as any instances of mislabeling. Fifty-two commercial products were collected from online and retail sources. DNA was extracted from each product in duplicate and tested for the presence of eight meat species (bovine, caprine, ovine, chicken, goose, turkey, porcine, and equine) using real-time polymerase chain reaction (PCR) with SYBR Green and species-specific primers. Of the 52 tested products, 31 were labeled correctly, 20 were potentially mislabeled, and 1 contained a non-specific meat ingredient that could not be verified. Chicken was the most common meat species found in the pet food products (n = 51), and none of the products tested positive for horsemeat. In three cases of potential mislabeling, one or two meat species were substituted for other meat species, but major trends were not observed. While these results suggest the occurrence of pet food mislabeling, further studies are needed to determine the extent of mislabeling and identify points in the production chain where mislabeling occurs.

Keywords

Pet foods, real-time PCR, meat species identification, mislabeling, adulteration, species substitution

1. Introduction

The pet food industry, including pet foods and other pet products and services, is a growing market in the United States. Over the past five years, U.S. pet industry expenditures have increased by approximately $10 billion, with close to $21 billion spent on pet food alone in 2012 (APPA, 2013). The U.S. Bureau of Labor Statistics (BLS) reports that nearly 75% of U.S. households own pets, totaling about 218 million pets, not including fish (Henderson, 2013). On average, each U.S. household spends more than $500 on pets annually, equating to about 1% of household expenditures.

The foods developed for pets are regulated by both federal and state entities. The U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) regulates animal feed and pet foods under the Federal Food, Drug, and Cosmetic Act (FFDCA). For product labeling standards, the FDA regulates product identification, net quantity, manufacturer's contact information, and the proper listing of ingredients (FDA, 2010). The Association of American Feed Control Officials (AAFCO), composed of state, federal, and international regulatory officials, is not a regulatory entity but has established a model of pet food regulations and guidelines that has been adopted by the FDA and many state regulatory offices. While it does not regulate the manufacturing of pet foods, the U.S. Department of Agriculture (USDA) regulates the interstate transportation and processing of animal products as well as the inspection of animal product imports and exports.

Although regulations exist for pet foods, increases in international trade and globalization of the food supply have amplified the potential for food fraud to occur. Food fraud is defined as "the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product, for economic gain," and it also greatly affects food safety and public health (Moore, Spink, & Lipp, 2012; Spink & Moyer, 2011, 2013). There are numerous possibilities for mislabeling and misidentification of meat species throughout the production chain, including at the abattoir, at meat and meat by-product processing plants, and at the food product manufacturing plant (Premanandh, 2013). The potential issues concerning meat and meat product authenticity include species misidentification, undeclared animal parts and ingredients, undeclared additives, and product origin (Montowska & Pospiech, 2011). Few studies have been published surveying meat species identification and mislabeling in processed foods for human consumption, let alone pet foods, suggesting a need for further research in this area. A South African study performed on species substitution and mislabeling of meat products reported that pork was the most commonly substituted meat, which poses a risk for Muslim and Jewish dietary restrictions (Cawthorn, Steinman, & Hoffman, 2013). In the same study, unapproved meat for human consumption—donkey, goat, and water buffalo—was detected in several of the tested processed and packaged meat products. Meat substitutions due to undeclared meat species were also detected in previous studies testing raw and cooked processed meat products for human consumption from the U.S., Turkey, Mexico, and Istanbul (Ayaz, Ayaz, & Erol, 2006; Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno, 2000; Hsieh, Woodward, & Ho, 1995; Ozpinar, Tezmen, Gokce, & Tekiner, 2013).

Processed meat products present a challenge in terms of food fraud detection, as meat species in these foods may be impossible to distinguish visually and may consist of a mixture of multiple species. For example, undeclared horsemeat was found in several Mexican hamburger and sausage products, as well as in raw meat samples from Turkey, which declared the products as beef (Ayaz et al., 2006; Flores-Munguia et al., 2000). With the recent discovery of horsemeat in ground meat products sold for human consumption in several European countries, the presence of horsemeat in U.S. consumer food and pet food products is also a concern (O'Mahony, 2013; Stanciu, Stanciuc, Dumitrascu, Ion, & Nistor, 2013). Considering the vast network in existence of global imports and exports, it is feasible that food fraud in one part of the world could spread elsewhere. One area where this possibility exists is in the cattle trade, for which the U.S. is the only major exporter that does not have a mandatory cattle traceability system or standards in place (Schroeder & Tonsor, 2012). Even though the USDA has implemented standards for animal disease traceability, the purpose of these standards is to only regulate and trace livestock moving interstate when diseased animals are found (USDA, 2013). The lack of a comprehensive cattle traceability system in the U.S. may increase the potential for meat species substitution and mislabeling (Shackell, 2008).

In addition to pet food mislabeling and food fraud, pet food safety is another area of concern, especially with commercialized pet foods that are specifically formulated to address immunological adverse food reactions (AFR). AFR are food allergies that may occur in both dogs and cats regardless of breed, sex, or age, causing chronic dermatological disorders and gastrointestinal diseases (Verlinden, Hesta, Millet, & Janssens, 2006; Vogelnest & Cheng, 2013). Some common food allergens in dogs and cats include meat proteins, such as beef and chicken (Raditic, Remillard, & Tater, 2011; Vogelnest & Cheng, 2013). AFR is typically diagnosed by an elimination diet, which limits the number of proteins in the diet and helps to identify the cause of the immunological response(s); the main treatment for AFR is to eliminate the cause of the reaction (Verlinden et al., 2006). Homemade diets are usually recommended, but commercial novel protein diets (NPD) and hydrolyzed protein diets (HPD) are also available on the market and usually contain one protein source; therefore, it is important that these pet food products are correctly labeled (Ricci et al., 2013; Verlinden et al., 2006). However, studies have shown that some NPD and HPD are mislabeled. In one study, undeclared mammalian and avian DNA and bone fragments were found in 10 of the 12 tested dry NPD and HPD products for dogs (Ricci et al., 2013). Another study found undeclared beef proteins in a dry dog food product listing venison as the only meat ingredient (Raditic et al., 2011). It is highly important to ensure that these pet food products on the market are safe and correctly labeled because incorrectly labeled products may cause elimination diets to fail and result in undiagnosed AFR in dogs and cats suffering from mild to severe chronic immunological response(s).

Meat species are commonly identified in foods using either DNA or protein analyses (Ballin, Vogensen, & Karlsson, 2009). Protein analyses, such as immunoassays, identify species through specific antigen-antibody interactions; however, they are limited to characterizing processed animal proteins (PAP) (Ballin et al., 2009). These proteins are challenging to analyze in certain processed foods because some proteins are specific to certain tissues and may not be found in a given product. In these circumstances, DNA-based methods, such as the polymerase chain reaction (PCR), are advantageous in that DNA is found in practically all tissues and is stable at higher temperatures (Ballin et al., 2009). The specific animal tissues contained in processed foods are sometimes unknown and are present in mixtures; therefore, DNA analyses are ideal in identifying meat species in highly processed foods (Ballin et al., 2009). Among DNA targets, mitochondrial DNA (mtDNA) is desirable in these food types because it is present at a higher copy number than chromosomal DNA and is therefore more likely to be detected during PCR (Ballin et al., 2009). One method that shows considerable promise for identification of meat species in heavily processed foods and feeds is real-time PCR (Yancy et al., 2009). This method is highly sensitive, rapid, and can be used to identify species in mixed products containing meat from multiple species.

The objective of this study was to perform a market survey of commercial canine and feline pet foods in order to identify the types of meat species present in these products as well as any instances of pet food mislabeling. This objective was accomplished using a real-time PCR assay targeting regions of mtDNA in eight different meat species.

2. Materials and Methods

2.1 *Sample collection and preparation*

A total of 52 commercial canine and feline pet food products representing a variety of meat species and processing methods were collected from retail stores in Orange County, California, and online stores in July and August 2013. Each pet food product was randomly assigned a unique three-digit sample identification number. The product's brand name, flavor or description, net weight, ingredient list, lot number, expiration date, place of origin, and purchase place and date were recorded. The USDA sample preparation and extraction standard protocols (Section 17.4) for the identification of animal species in meat and poultry products were used for the pet food sample preparation, with a few modifications (USDA, 2005). Sterileware scoops (Scienceware, Wayne, NJ) or flame-sterilized tweezers were used to aseptically remove 30.0 g of dry food products or treats that were placed into 24 oz. Whirl-Pak® Stand-up bags (Nasco, Fort Atkinson, WI) with 60.0 mL of sterile water. The products were incubated at room temperature for 1 h and then processed in a Seward Stomacher® 400 Circulator (Seward USA, Port Saint Lucie, FL) at 230 rpm for 60 s. The entire contents of wet food products were placed in 7 oz. Whirl-Pak® Write-on bags (Nasco, Fort Atkinson, WI) and the bags were hand-mixed for 60 s to homogenize the samples. Two ~10 mg subsamples were collected from each product for DNA extraction.

2.2 *DNA extraction and PCR preparation*

The DNA extraction portion of the Extract-N-Amp Tissue PCR Kit (#XNAT2; Sigma-Aldrich, St. Louis, MO) was used to extract the DNA in duplicate from each sample using half the volumes suggested by the manufacturer. Aliquots of 50.0 µL of Extraction solution and 12.5 µL of Tissue Preparation solution were added to each tube containing a tissue subsample. A reagent blank was included with each DNA extraction as a negative control, and the samples were incubated at 55ºC for 10 min, and then at 95ºC for an additional 3 min. After both incubations, 50.0 µL of Neutralization Solution B was added to each sample, and then the samples were centrifuged at 13,000 rpm for 1 min. The supernatant was carefully removed avoiding the lipid layer when present and without disturbing the pelleted debris. The extracted supernatant for each sample was then used as the extracted DNA template for real-time PCR. The quantity and quality of starting DNA was not determined, as DNA extracted with this method is a crude extract that could not be accurately measured with a spectrophotometer (Hellberg, Kawalek, Van, Shen, & Williams-Hill, 2014).

2.3 *Real-Time PCR*

All real-time PCR amplification reactions were performed with the Rotor-Gene® Q (RGQ) Real-Time PCR Cycler and software (Qiagen, Germantown, MD) and contained 12.5 µL of iQ™ SYBR® Green Supermix (2X) (Bio-Rad, Hercules, CA), 1.0 µL of each oligonucleotide primer (forward and reverse), 8.5 µL of sterile water, and 2.0 µL of extracted DNA or control for a total reaction volume of 25.0 µL. All samples were tested for the presence of eight animal species (bovine, caprine, ovine, avian [chicken, goose, turkey], porcine, and equine) using species-specific primers described in previous studies (Kesmen, Sahin, & Yetim, 2007; Yancy et al., 2009). The final primer concentrations in each PCR reaction were 0.16 µM for bovine, 0.25 µM for caprine and ovine, 0.2 µM for avian, and 0.3 µM for porcine and equine. Each PCR run included the reagent blank from the DNA extraction, a no-template control, and a positive control DNA. For the positive control, three 10-fold serial dilutions of DNA for each meat species were made using Tris-EDTA buffer, pH 8.0 (E112-100ml; BioExpress, Kaysville, UT) and were included in each PCR run. Thermocycling settings for bovine, caprine, ovine, and avian were carried out as described in Yancy et al. (2009) with an initial incubation at 94ºC for 2 min and then 50 cycles of 94ºC for 10 s, 58.9ºC for 15 s, and 72ºC for 40 s, with a single fluorescent reading taken at the end of each cycle. The porcine and equine thermocycling conditions included an initial incubation at 92ºC for 2 min and then 35 cycles of 94ºC for 50 s, 55ºC for 50 s (porcine) or 62ºC for 50 s (equine), and 72ºC for 60 s with a single fluorescent reading taken at the end of each cycle and a final extension at 72ºC for 5 min. These conditions were taken from the protocol originally described by Kesmen et al. (2007) for use with conventional PCR and were only used after sensitivity testing showed the conventional and real-time PCR results to be equivalent. A melt-curve analysis was completed at the end of each run for all meat species tested to confirm the specificity of amplification. Both the threshold cycle (Ct) and melt-curve values and threshold were set manually by comparison with positive controls. Results were determined to be positive if at least one of the subsamples tested met the criteria of (1) having a Ct value for the meat species being tested and (2) having a melting temperature within 0.5 ºC of the average positive control melting temperatures for that run. Results were qualitative and reported in terms of presence or absence of a given species. In cases where a declared species was found to be absent, additional testing was carried out to address the possibility of false negatives. Each of these samples was re-extracted and re-tested in duplicate. These samples were also tested with positive control tissue spikes to account for possible inhibitors in the sample matrix. Positive control tissue of the declared but not detected species was mixed with the pet food sample at levels of 1%, 5%, and 10%. These spiked samples were then extracted using the Extract-N-Amp Tissue PCR Kit and tested with real-time PCR, as described above. All spiking tests were also carried out in duplicate.

2.4 *Statistical analyses*

The rate of potentially mislabeled products was statistically compared across pet food categories using IBM SPSS Statistics 21 (Armonk, NY). The rate of potentially mislabeled dog food products was compared to the rate of potentially mislabeled cat food products using a Pearson's chi-square test, with a pre-determined 2-sided significance value of $p < 0.05$. The rate of potentially mislabeled dry foods, wet foods, and treats was compared using a Fisher's exact test, with a predetermined 2-sided significance value of $p < 0.017$ (0.05/3) based on the Bonferroni correction for multiple tests.

3. Results and Discussion

3.1 *Meat species detected in pet foods*

Meat species were identified and analyzed in all 52 commercial canine and feline pet food products and treats collected for this study (Table 1). Some of the tested meat species in this study were detected in many products while other meat species were detected in few or none of the products. Of the eight meat species tested, chicken was the most commonly detected meat, with 51 of the products testing positive (Fig. 1). The lower costs of chicken when compared to beef or pork may explain, in part, why chicken was the most common meat ingredient detected in the pet foods tested (NCC, 2012). Although the wholesale and retail prices of beef, pork, and chicken have increased every year since 1960, the 2012 wholesale and retail prices of chicken per pound were approximately 35% and 25% lower than wholesale and retail beef prices, respectively (NCC, 2012). The 2012 wholesale and retail prices for pork were between those for beef and chicken (NCC, 2012). Pork was the second most common meat species detected, with positive identifications for 35 products, and beef, turkey, and lamb were detected in 34, 32, and 26 products, respectively (Fig. 1). Goat and goose were detected sparingly in a few products containing non-specific meat ingredients (e.g., animal fat, meat and bone meal, animal digest); however, they were not specifically labeled as an ingredient in any of the tested pet food products.

With the general lack of meat authentication testing and the recent food fraud and horsemeat scandal in Europe, finding horsemeat in U.S. consumer food and pet food products is a concern (O'Mahony, 2013; Premanandh, 2013). Due to the ability to detect low levels of horsemeat in processed food products (Kesmen et al., 2007), each pet food product in this study was tested for equine DNA; however, all of the tested pet food products were negative (Table 1). This finding suggests that horsemeat was not incorporated nor used as a meat substitute in any of the tested pet food products (n = 52), including in non-specific meat ingredients.

More than half of the pet food products tested (n = 38) contained one or more non-specific meat ingredient(s) (Table 1). Of those products, animal or poultry fat, meat by-products, meat and bone meal (MBM), animal digest, and poultry by-product meal were the most common non-specific meat ingredients listed on the product labels. The pet food industry has a large demand for animal by-products, and hog (porcine) and steer (bovine) by-product values have increased since 2000 (Marti, D. L., Johnson, R. J., & Mathews, K. H., Jr., 2011). The value of porcine by-products has increased 80.3% between 2000 and 2010, and the value of bovine by-products has risen 34.8% during the same time frame (Marti et al., 2011). Because of its use in pet foods and in the medical industry, and with a rising demand on exports, animal by-product use has increased over the years (Marti et al., 2011). Twenty-five products (14 dry foods and 11 pet treats) contained "animal fat" or "poultry fat" as an ingredient (Table 2), which is defined as the fatty acid product from commercially rendered, extracted mammalian or poultry animal tissue, respectively (AAFCO, 2013). Chicken was the most common species detected in these products (Table 2), which may be expected considering the lower wholesale and retail prices for chicken compared to those for beef and pork, as discussed above. Pork was the second most common meat species detected in these products and the most common mammalian meat species detected in products containing "animal fat" specifically. On the other hand, goose was the least common meat species and detected in only one product that listed animal fat as its ingredient.

The ingredients "meat by-product" or "dried meat by-product," which are the clean and non-rendered parts derived from mammals that are not considered meat or meat flesh (AAFCO, 2013), were included in 11 of the products tested (Table 2). Nine out of the eleven products containing meat by-products as an ingredient were wet pet foods, and the other two were treats. The most common detected species was pork, found in five of the 11 products. Five products (4 dry pet foods and 1 treat) listed MBM as an ingredient, which is considered the rendered meat parts and bones from mammals (Table 2) (AAFCO, 2013). All of these products contained at least two mammalian meat species, while one contained all four mammalian meat species (bovine, caprine, ovine, and porcine). Additionally, "animal digest," defined as the clean and un-decomposed animal tissues that have been obtained through chemical and/or enzymatic hydrolysis, was included as an ingredient in five of the tested pet food products, all of which were dry pet foods (Table 2) (AAFCO, 2013). Beef and chicken were detected in all of these products, whereas turkey and pork were detected in four of the products, lamb was detected in three of the products, and one product contained caprine meat. Poultry by-product meal consisting of the ground, rendered, and clean parts of poultry was listed as an ingredient in four of the tested dry pet food products (Table 2) (AAFCO, 2013). Both chicken and turkey were found in all products listing "poultry by-product meal" as an ingredient, while goose was not detected in any product containing poultry by-product meal.

3.2 *Pet food mislabeling*

Of the 52 products tested, 31 were found to be labeled correctly, meaning that all meat species included on the product label were detected in the sample, and undeclared meat species were not detected (Table 1). Twenty products were considered potentially mislabeled because they either (1) contained meat species that were not included on the product label and/or (2) did not contain meat species that were included on the product label. Labeling of one product (P011, wet cat food) listing "meat by-products" as an ingredient could not be verified because none of the five tested mammalian meat species were detected in the product. It is possible that the meat by-product ingredient contained other untested mammalian meat species. Another product, P016 (wet cat food), listed an animal species not tested in this study (i.e., venison) as an ingredient. Although the presence of venison could not be verified, the product was deemed potentially mislabeled based on the possible substitution of turkey and pork for beef and lamb (Table 1).

Of the 20 potentially mislabeled products, 13 were dog food and 7 were cat food; however, this difference was not statistically significant, according to a chi-square test (2-sided p-value > 0.05). In comparing wet food, dry food, and treats, the rate of potentially mislabeled wet food products (n = 12/16) was found to be significantly higher than the rate of potentially mislabeled dry food products (n = 2/17), according to a Fisher's exact test with the Bonferroni correction (p value < 0.017). However, there were no significant differences between the rate of potentially mislabeled treats (n = 6/18) and the rate of potentially mislabeled wet or dry foods. Overall, these results indicate a higher frequency of mislabeling in wet foods compared to dry foods for the sample set analyzed in this study. Interestingly, half of the potentially mislabeled wet food products (n = 6) included one non-specific meat ingredient, whereas only one potentially mislabeled treat product listed a non-specific meat ingredient and none of the potentially mislabeled dry food products listed a non-specific meat ingredient.

Instances where meat species were included on the product's label but were not detected in the product occurred in seven of the 20 potentially mislabeled products, with bovine being the most common declared but undetected meat species (Table 1). These seven samples were subjected to spiking tests with positive control tissue to address the possibility of false negatives due to inhibition from the sample matrix. The results of the spiking tests with each product showed that the assay was able to detect tissue from pork, lamb and chicken at levels as low as 1% in all the sample matrices tested, and that turkey and beef could be detected at levels as low as 1–5%, depending on the product. For example, among the four products with declared but undetected beef, one wet cat food product (P016) and one dog treat (P035) showed a detection limit for beef of 1%, whereas two wet dog food products (P002 and P004) showed a detection limit for beef of 5%. In three of the four products, beef was listed as either the first or second ingredient and also appeared later in the ingredient list, suggesting that detection should have been possible if the species was indeed present. Taken together, these results indicate that the seven products with declared but undetected species either (1) did not contain the declared meat species or (2) contained the declared meat species at levels below the detection limit for this assay.

Meat species that were not included on the product label were detected in 16 of the 20 potentially mislabeled products, with pork being the most common undeclared meat species detected (Table 1). For example, product P019 (dry dog food) was found to contain undeclared ovine, turkey, and porcine ingredients in addition to the declared chicken and bovine ingredients. In another instance with a cat treat product (P045), undeclared pork was detected in addition to the declared chicken ingredients. Interestingly, in three cases, one to two meat species were substituted for other meat species listed on the label. These included instances of undeclared pork in place of beef in a wet dog food product (P002), undeclared turkey and pork in place of beef and lamb in a wet cat food product (P016), and undeclared chicken in place of beef and pork in a dog treat product (P035) (Table 1). Taken together, these results indicate a possible trend for the substitution of lower-cost ingredients, such as poultry meats, for higher cost ingredients, such as beef and lamb (Mundi, 2014; Raditic et al., 2011), although more research would be needed to verify this trend.

For six products, meat species emphasized in the product name and/or description on the front of the product packaging was not detected in the product. This occurred in four wet pet foods and two pet treats, in which three of the products were for dogs and three for cats. The declared but undetected meat species were beef, lamb, pork, and turkey, with beef being the most common. Including a meat species in the product name when it is not actually detectable in the product itself could be considered to be misleading according to the labeling requirements set forth by the AAFCO model regulations for product naming (FDA, 2010). AAFCO's "flavor rule" states that a sufficient amount of the meat or substance(s) that characterizes the meat flavor must be used to avoid the product from being misleading (FDA, 2010). Product P002 (wet dog food) listed "beef" in its product flavor description, and included deboned beef and beef broth as its first two ingredients, respectively; however, bovine DNA was not detected in this product (Table 1). Instead, pork was detected, indicating a possible meat substitution and a potentially misleading product to consumers (Table 1). Another example was product P017 (wet cat food), which listed "turkey" in its product flavor description and as its third ingredient, but turkey DNA was not detected in the product. This product contained non-specific meat ingredients; however, of the eight meat species tested, chicken and goat were the only meat species detected (Table 1). Product P035 (dog treats) listed both "bacon and beef" in its product description and did not include any non-specific meat ingredients; however, neither porcine nor bovine DNA were detected in the product. Instead, chicken was the only meat species detected in product P035 (Table 1). These products could potentially be misleading to consumers and may pose a risk to pets with AFR to certain meat proteins.

Similar to the findings of the current study, previous market studies have also found a number of meat products to be mislabeled (Ayaz et al., 2006; Cawthorn et al., 2013; Flores-Munguia et al., 2000; Hsieh et al., 1995; Ozpinar et al., 2013; Raditic et al., 2011), and pork has been found to be a commonly undeclared but detected ingredient. For example, in the South African study mentioned previously, 68% of processed and packaged meat products for human consumption were found to contain undeclared plant and/or animal species, with pork being the most common undeclared animal species (Cawthorn et al., 2013). In several processed meat samples tested in Istanbul, undeclared horse, pork, and chicken meat were detected (Ozpinar et al., 2013). It was also found that pork was substituted for beef, chicken was a substitute for pork-based sausages, and over half (53.4%) of samples were mislabeled (Ozpinar et al., 2013). In a U.S. study conducted in Florida, meat substitution was detected in 16.6% of samples, with incidences of mislabeling occurring more in cooked ground meat than in raw ground meat products (Hsieh et al., 1995). The study also found that sheep, pork, and poultry were the most common undeclared meat species. Furthermore, in a study conducted in Mexico, some samples of hamburger and sausage meat contained undeclared equine and porcine meat species (Flores-Munguia et al., 2000). Many of the cooked or fermented sausages and ground meat products collected in Turkey contained undeclared meat species, such as cooked "beef-only" samples containing poultry meat and raw "beef" samples containing horse and deer meat (Ayaz et al., 2006). The results of these studies combined with the current study indicate that meat species substitution and adulteration occurs in processed foods intended for either human or animal consumption. Some potential factors contributing to this mislabeling trend may be (1) intentional substitution with cheaper alternative meat species for economic gain or (2) unintentional substitution caused by accidental cross-contamination in the production chain.

While a seemingly high percentage of pet foods were found to be potentially mislabeled in this study, the manner in which mislabeling occurred is not clear. For example, it is unknown as to whether the mislabeling was intentional or accidental and at which point(s) in the production chain it took place. Real-time PCR is a sensitive assay that is capable of picking up on low levels of DNA in a product. For example, the real-time PCR assay developed by Yancy et al. (2009) was reported to be capable of identifying species in animal feeds at levels as low as 0.1%. In manufacturing and processing plants that handle more than one meat species on the same equipment, some animal tissue may remain and contaminate the next product during processing and handling, especially in instances where the equipment is not thoroughly cleaned and sanitized between product lines (Premanandh, 2013). Another possible reason for the mislabeling observed is due to a lack of traceability from the farm to the final food product

(Shackell, 2008), which may allow for intentional or unintentional substitution of one animal product for another to go unnoticed or undocumented.

4. Conclusion

Although there are pet food regulations in place in the United States that are enforced by federal and state entities, there is still a lack of information on meat species authentication as well as accidental mislabeling and intentional food fraud. To date, few studies have been published on the prevalence of meat species mislabeling in pet foods. While this study suggests the occurrence of pet food mislabeling on the commercial market, further studies are needed to determine the extent of mislabeling and to identify points in the production chain where mislabeling occurs. Future areas of work also include the expansion of the tested meat species to include seafood and uncommon meat species that have been detected in mislabeled products for human consumption and testing of products marketed for pets that suffer from AFR, such as those that claim to contain no animal proteins, commercial novel proteins and/or hydrolyzed proteins.

Acknowledgements

The authors gratefully thank Chapman University Schmid College of Science and Technology for their support and Taylor Patti for assisting with real-time PCR laboratory work.

References

AAFCO. (2013). *Association of American Feed Control Officials. 2013 Official Publication*.

APPA. (2013). American Pet Products Association. U.S. Pet Industry Market Size & Ownership Statistics.

Ayaz, Y., Ayaz, N. D., & Erol, I. (2006). Detection of species in meat and meat products using enzyme-linked immunosorbent assay. *Journal of Muscle Foods, 17*(2), 214-220.

Ballin, N. Z., Vogensen, F. K., & Karlsson, A. H. (2009). Species determination - Can we detect and quantify meat adulteration? *Meat Science, 83*(2), 165-174.

Cawthorn, D. M., Steinman, H. A., & Hoffman, L. C. (2013). A high incidence of species substitution and mislabelling detected in meat products sold in South Africa. *Food Control, 32*(2), 440-449.

FDA. (2010). Food and Drug Administration. Animal and Veterinary Section: Pet Food Labels - General. Accessible at: http://www.fda.gov/AnimalVeterinary/ResourcesforYou/ucm047113.htm. Accessed 3/24/2014.

Flores-Munguia, M. E., Bermudez-Almada, M. C., & Vazquez-Moreno, L. (2000). A research note: Detection of adulteration in processed traditional meat products. *Journal of Muscle Foods, 11*(4), 319-325.

Hellberg, R. S., Kawalek, M. D., Van, K. T., Shen, Y., & Williams-Hill, D. M. (2014). Comparison of DNA extraction and PCR setup methods for use in high-throughput DNA barcoding of fish species. *Food Analytical Methods*. Advance online publication. doi:10.1007/s12161-014-9865-z

Henderson, S. (2013). Spending on pets: "Tails" from the Consumer Expenditure Survey. *Beyond the Numbers: Prices & Spending, 2*(16).

Hsieh, Y. H. P., Woodward, B. B., & Ho, S. H. (1995). Detection of species substitution in raw and cooked meats using immunoassays. *Journal of Food Protection, 58*(5), 555-559.

Kesmen, Z., Sahin, F., & Yetim, H. (2007). PCR assay for the identification of animal species in cooked sausages. *Meat Science, 77*, 649-653.

Marti, D. L., Johnson, R. J., & Mathews, K. H., Jr. (2011). Where's the (not) meat? Byproducts from beef and pork production. In: USDA Economic Research Service.

Montowska, M., & Pospiech, E. (2011). Authenticity determination of meat and meat products on the protein and DNA basis. *Food Reviews International, 27*(1), 84-100.

Moore, J. C., Spink, J., & Lipp, M. (2012). Development and application of a database of food ingredient fraud and economically motivated adulteration from 1980 to 2010. *Journal of Food Science, 77*(4), R118-R126.

Mundi, I. (2014). Lamb monthly price - U.S. cents per pound. Accessible at: http://www.indexmundi.com/commodities/?commodity=lamb&months=300. Accessed 3/24/2014.

NCC. (2012). The National Chicken Council. Wholesale and Retail Prices for Chicken, Beef, and Pork | The National Chicken Council. Accessible at: http://www.nationalchickencouncil.org/about-the-industry/statistics/wholesale-and-retail-prices-for-chicken-beef-and-pork/. Accessed 3/24/2014.

O'Mahony, P. J. (2013). Finding horse meat in beef products-a global problem. *Qjm-an International Journal of Medicine, 106*(6), 595-597.

Ozpinar, H., Tezmen, G., Gokce, I., & Tekiner, I. H. (2013). Detection of Animal Species in Some Meat and Meat Products by Comparatively Using DNA Microarray and Real Time PCR Methods. *Kafkas Universitesi Veteriner Fakultesi Dergisi, 19*(2), 245-252.

Premanandh, J. (2013). Horse meat scandal - A wake-up call for regulatory authorities. *Food Control, 34*(2), 568-569.

Raditic, D. M., Remillard, R. L., & Tater, K. C. (2011). ELISA testing for common food antigens in four dry dog foods used in dietary elimination trials. *Journal of Animal Physiology and Animal Nutrition, 95*(1), 90-97.

Ricci, R., Granato, A., Vascellari, M., Boscarato, M., Palagiano, C., Andrighetto, I., Diez, M., & Mutinelli, F. (2013). Identification of undeclared sources of animal origin in canine dry foods used in dietary elimination trials. *Journal of Animal Physiology and Animal Nutrition, 97*, 32-38.

Schroeder, T. C., & Tonsor, G. T. (2012). International cattle ID and traceability: Competitive implications for the US. *Food Policy, 37*, 31-40.

Shackell, G. H. (2008). Traceability in the meat industry - the farm to plate continuum. *International Journal of Food Science and Technology, 43*(12), 2134-2142.

Spink, J., & Moyer, D. C. (2011). Defining the public health threat of food fraud. *Journal of Food Science, 76*(9), R157.

Spink, J., & Moyer, D. C. (2013). Understanding and combating food fraud. *Food Technology*(1), 30.

Stanciu, S., Stanciuc, N., Dumitrascu, L., Ion, R., & Nistor, C. (2013). The effects of horse meat scandal on Romanian meat market. *Cross-Cultural Management Journal, 27*(1), 174.

USDA. (2005). United States Department of Agriculture. Identification of Animal Species in Meat and Poultry Products. Food Safety Inspection Service, Office of Public Health Science, MLG 17.02.

USDA. (2013). United States Department of Agriculture. Animal Disease Traceability: General Standards, v2.2. Accessible at:

http://www.aphis.usda.gov/traceability/downloads/ADT_standards.pdf. Accessed 3/24/2014.

Verlinden, A., Hesta, M., Millet, S., & Janssens, G. P. J. (2006). Food allergy in dogs and cats: A review. *Critical Reviews in Food Science and Nutrition, 46*(3), 259-273.

Vogelnest, L. J., & Cheng, K. Y. (2013). Cutaneous adverse food reactions in cats: retrospective evaluation of 17 cases in a dermatology referral population (2001-2011). *Australian Veterinary Journal, 91*(11), 443-451.

Yancy, H. F., Washington, J. D., Callahan, L., Mason, J. A., Deaver, C. M., Farrell, D. E., Ha, T. A. I., Sespico, E., Falmlen, D., & Myers, M. J. (2009). Development, evaluation, and peer verification of a rapid real-time PCR method for the detection of animal material. *Journal of Food Protection, 72*(11), 2368-2374.

Figure Captions.

Figure 1. Number of products (n = 52) containing the tested meat species.

Table 1. Results of meat species identification in pet food products and treats by real-time PCR.

Sample No.	Product Type	Meat Ingredients	Meat Species							
			Bovine (beef)	**Caprine (goat)**	**Ovine (lamb)**	**Chicken (avian)**	**Goose (avian)**	**Turkey (avian)**	**Porcine (pork)**	**Equine (horse)**
P001	Dog food (wet)	Beef by-products Liver (beef) Meat by-products Chicken Chicken by-products	+	–	–	+	–	–	–	–
P002[a]	Dog food (wet)	Deboned beef Beef broth	– [b]	–	–	–	–	–	+ [c]	–
P003[a]	Dog food (wet)	Chicken broth Chicken Turkey Beef Chicken liver Beef liver Lamb	+	–	+	+	–	– [b]	–	–
P004[a]	Dog food (wet)	Beef Liver (beef) Meat by-products Turkey	– [b]	–	–	–	–	+	+	–
P005[a]	Dog food (wet)	Liver (lamb) Lamb Meat by-products Turkey	–	–	+	+ [c]	–	+	+	–
P006[a]	Dog food (wet)	Pork Liver (pork) Chicken Meat by-products	+	–	–	+	–	+ [c]	+	–
P007	Dog food (wet)	Meat broth Beef Pork liver Ham (pork) Animal plasma	+	–	–	+	–	+	+	–
P008[a]	Dog food (wet)	Turkey Pork liver Pork plasma Chicken fat	+ [c]	–	–	+	–	+	+	–
P009	Cat food (wet)	Poultry broth Turkey Liver (turkey) Meat by-products Chicken	+	–	–	+	–	+	–	–

Sample No.	Product Type	Meat Ingredients	Meat Species: Bovine (beef)	Caprine (goat)	Ovine (lamb)	Chicken (avian)	Goose (avian)	Turkey (avian)	Porcine (pork)	Equine (horse)
P010[a]	Cat food (wet)	Meat broth Chicken Meat by-products Chicken by-products Lamb	–	–	+	+	–	+ [c]	–	–
P011[d]	Cat food (wet)	Meat by-products Chicken Poultry by-products	–	–	–	+	–	+	–	–
P012	Cat food (wet)	Chicken Chicken broth Beef Chicken fat	+	–	–	+	–	–	–	–
P013[a]	Cat food (wet)	Pork Pork broth Pork liver	+ [c]	–	–	+ [c]	–	–	+	–
P014[a]	Cat food (wet)	Chicken Chicken liver Pork by-products	+ [c]	–	–	+	–	–	+	–
P015[a]	Cat food (wet)	Chicken Turkey giblets Meat by-products Liver (chicken) Chicken fat	+	–	–	+	–	– [b]	–	–
P016[a]	Cat food (wet)	Beef Beef broth Beef liver Lamb liver Venison Lamb Chicken meal	– [b]	–	– [b]	+	–	+ [c]	+ [c]	–
P017[a]	Cat food (wet)	Liver (turkey) Turkey Meat by-products Chicken	–	+	–	+	–	– [b]	–	–
P018	Dog food (dry)	Meat & bone meal Animal fat	+	–	+	+	–	+	–	–
P019[a]	Dog food (dry)	Chicken Chicken meal Beef fat	+	–	+ [c]	+	–	+ [c]	+ [c]	–

Sample No.	Product Type	Meat Ingredients	Meat Species							
			Bovine (beef)	**Caprine (goat)**	**Ovine (lamb)**	**Chicken (avian)**	**Goose (avian)**	**Turkey (avian)**	**Porcine (pork)**	**Equine (horse)**
P020	Dog food (dry)	Chicken by-product meal Beef tallow Beef Animal fat	+	+	+	+	–	+	+	–
P021	Dog food (dry)	Beef & bone meal Animal fat Animal digest	+	–	–	+	–	–	+	–
P022	Dog food (dry)	Chicken by-product meal Animal fat Beef Meat & bone meal Animal digest	+	–	+	+	–	+	+	–
P023[a]	Dog food (dry)	Lamb meal Poultry fat	+ [c]	–	+	+	–	+	+ [c]	–
P024	Dog food (dry)	Meat & bone meal Animal fat	+	+	+	+	–	+	+	–
P025	Dog food (dry)	Chicken by-product meal Animal fat Chicken	–	–	+	+	–	+	–	–
P026	Dog food (dry)	Beef Animal fat Poultry by-product meal Animal digest	+	–	+	+	–	+	+	–
P027	Cat food (dry)	Chicken Chicken meal Animal fat	+	–	+	+	–	+	+	–
P028	Cat food (dry)	Chicken Chicken by-product meal Chicken meal Animal fat	–	+	+	+	–	+	+	–
P029	Cat food (dry)	Chicken meal Animal fat Chicken	+	–	+	+	–	+	+	–
P030	Cat food (dry)	Poultry by-product meal Animal fat Animal digest Chicken meal Turkey by-product meal	+	+	–	+	–	+	+	–

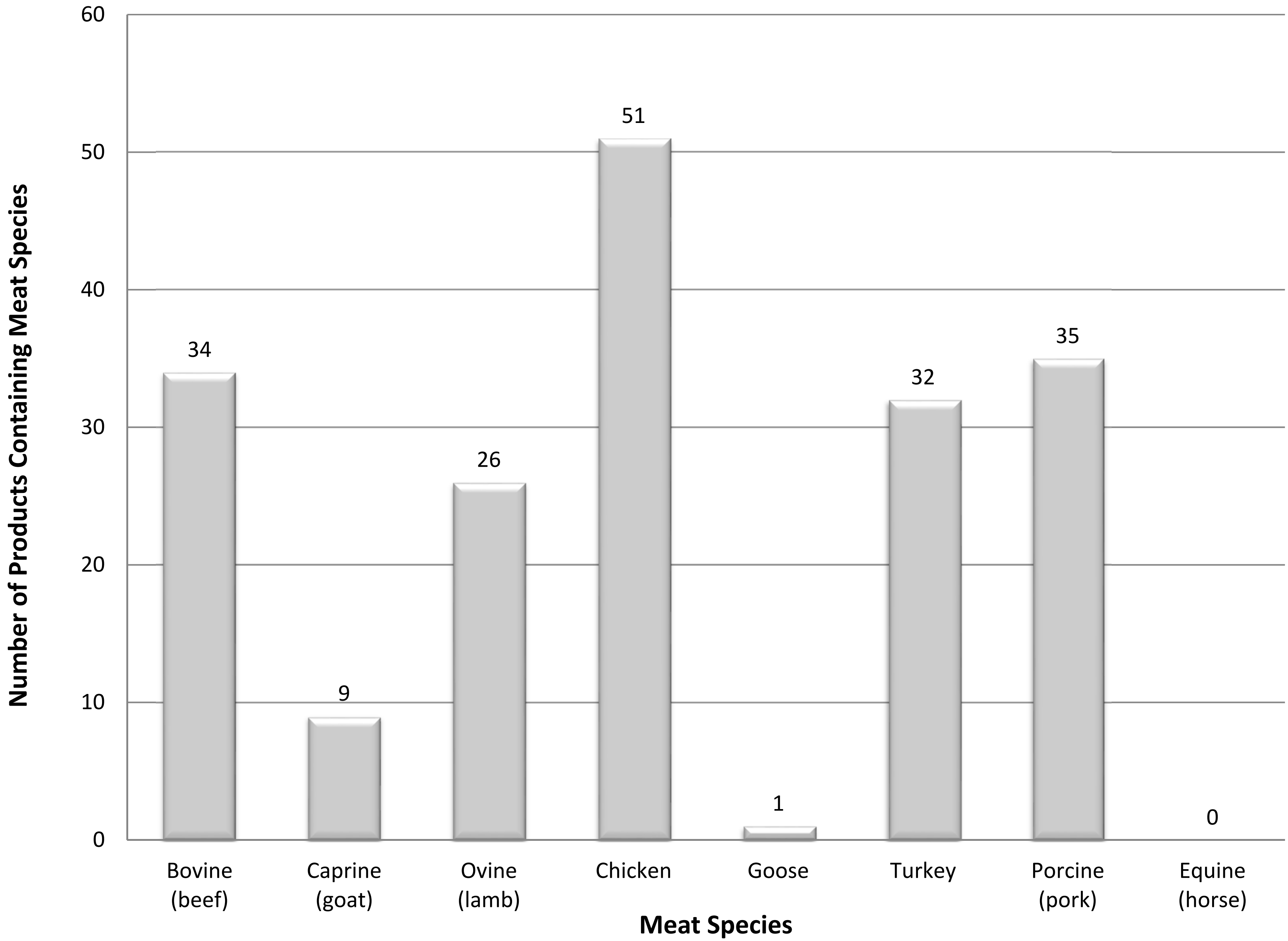
Sample No.	Product Type	Meat Ingredients	Meat Species Bovine (beef)	Caprine (goat)	Ovine (lamb)	Chicken (avian)	Goose (avian)	Turkey (avian)	Porcine (pork)	Equine (horse)
P031	Cat food (dry)	Chicken by-product meal Beef tallow Animal digest Turkey by-product meal	+	–	+	+	–	+	–	–
P032	Cat food (dry)	Poultry by-product meal Animal fat Chicken	+	–	+	+	–	–	+	–
P033	Cat food (dry)	Chicken by-product meal Meat & bone meal Beef tallow Turkey by-product meal	+	–	+	+	–	+	+	–
P034	Cat food (dry)	Poultry by-product meal Animal fat	+	–	–	+	–	+	+	–
P035[a]	Dog treats	Bacon Bacon fat Beef	– [b]	–	–	+ [c]	–	–	– [b]	–
P036[a]	Dog treats	Chicken	+ [c]	–	–	+	–	–	–	–
P037[a]	Dog treats	Chicken Beef	+	–	+ [c]	+	–	+ [c]	+ [c]	–
P038	Dog treats	Lamb Poultry fat	–	–	+	+	–	+	–	–
P039[a]	Dog treats	Meat & bone meal Beef fat	+	+	–	+ [c]	–	+ [c]	–	–
P040	Dog treats	Chicken by-product meal Beef Animal fat	+	–	+	+	–	–	–	–
P041[a]	Dog treats	Beef Beef by-products Beef liver	+	–	–	+ [c]	–	–	+ [c]	–
P042	Dog treats	Beef Liver Animal fat Chicken by-product meal	+	+	+	+	–	+	+	–
P043	Dog treats	Bacon Dried bacon fat Animal fat	+	+	+	+	–	–	+	–
P044	Dog treats	Chicken Animal fat	–	+	+	+	–	+	–	–
P045[a]	Cat treats	Chicken Chicken meal	–	–	–	+	–	–	+ [c]	–

Sample No.	Product Type	Meat Ingredients	Meat Species							
			Bovine (beef)	Caprine (goat)	Ovine (lamb)	Chicken (avian)	Goose (avian)	Turkey (avian)	Porcine (pork)	Equine (horse)
P046	Cat treats	Chicken	–	–	–	+	–	–	–	–
P047	Cat treats	Chicken meal Animal fat Chicken	–	–	–	+	–	–	+	–
P048	Cat treats	Chicken meal Animal fat	–	–	–	+	–	–	+	–
P049	Cat treats	Chicken meal Animal fat Bacon	+	–	+	+	–	+	+	–
P050	Cat treats	Animal liver Chicken by-product meal Animal fat	–	–	–	+	–	–	+	–
P051	Cat treats	Chicken meal Animal fat Dried meat by-products	–	–	+	+	–	–	+	–
P052	Cat treats	Chicken by-product meal Animal fat Dried meat-by products	–	–	+	+	+	+	+	–

[a] Potentially mislabeled.
[b] Meat species listed on the product label was not detected.
[c] Contains undeclared meat species.
[d] Labeling could not be confirmed.

Table 2. Meat species detected in products (n = 38) with non-specific meat ingredients on the label.

Non-specific meat ingredients on label	Number of products containing each meat species							
	Bovine (beef)	**Caprine (goat)**	**Ovine (lamb)**	**Chicken**	**Goose**	**Turkey**	**Porcine (pork)**	**Equine (horse)**
"Animal fat" or "Poultry fat" (n = 25)	16	7	19	25	1	17	20	0
"Meat by-product" or "Dried meat by-product" (n = 11)	4	1	4	—	—	—	5	0
"Meat & bone meal" (n = 5)	5	2	4	—	—	—	3	0
"Animal digest" (n = 5)	5	1	3	5	0	4	4	0
"Poultry by-product meal" (n = 4)	—	—	—	4	0	4	—	—



Number of Products Containing Meat Species
60
50
40
30
20
10
0
34
9
26
51
1
32
35
0
Bovine (beef)
Caprine (goat)
Ovine (lamb)
Chicken
Goose
Turkey
Porcine (pork)
Equine (horse)
Meat Species