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Identification of meat species in pet foods using a real-time polymerase chain reaction (PCR) assay

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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.

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

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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 analyzes (Ballin, Vogensen, & Karlsson, 2009). Protein analyzes, 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 analyzes 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