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

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1   **Identification of Meat Species in Pet Foods Using a Real-time Polymerase Chain Reaction**

2   **(PCR) Assay**

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15 **Abstract**

16 Product mislabeling, adulteration, and substitution are increasing concerns in highly processed

17 foods, including pet foods. Although regulations exist for pet foods, there is currently a lack of

18 information on the prevalence of pet food mislabeling. The objective of this study was to

19 perform a market survey of pet foods and pet treats marketed for domestic canines and felines to

20 identify meat species present as well as any instances of mislabeling. Fifty-two commercial

21 products were collected from online and retail sources. DNA was extracted from each product in

22 duplicate and tested for the presence of eight meat species (bovine, caprine, ovine, chicken,

23 goose, turkey, porcine, and equine) using real-time polymerase chain reaction (PCR) with SYBR

24 Green and species-specific primers. Of the 52 tested products, 31 were labeled correctly, 20 were

25 potentially mislabeled, and 1 contained a non-specific meat ingredient that could not be verified.

26 Chicken was the most common meat species found in the pet food products ($n = 51$), and none of

27 the products tested positive for horsemeat. In three cases of potential mislabeling, one or two

28 meat species were substituted for other meat species, but major trends were not observed. While

29 these results suggest the occurrence of pet food mislabeling, further studies are needed to

30 determine the extent of mislabeling and identify points in the production chain where

31 mislabeling occurs.

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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

61 economic gain," and it also greatly affects food safety and public health (Moore, Spink, & Lipp,

62 2012; Spink & Moyer, 2011, 2013). There are numerous possibilities for mislabeling and

63 misidentification of meat species throughout the production chain, including at the abattoir, at

64 meat and meat by-product processing plants, and at the food product manufacturing plant

65 (Premanandh, 2013). The potential issues concerning meat and meat product authenticity include

66 species misidentification, undeclared animal parts and ingredients, undeclared additives, and

67 product origin (Montowska & Pospiech, 2011). Few studies have been published surveying meat

68 species identification and mislabeling in processed foods for human consumption, let alone pet

69 foods, suggesting a need for further research in this area. A South African study performed on

70 species substitution and mislabeling of meat products reported that pork was the most commonly

71 substituted meat, which poses a risk for Muslim and Jewish dietary restrictions (Cawthorn,

72 Steinman, & Hoffman, 2013). In the same study, unapproved meat for human consumption—

73 donkey, goat, and water buffalo—was detected in several of the tested processed and packaged

74 meat products. Meat substitutions due to undeclared meat species were also detected in previous

75 studies testing raw and cooked processed meat products for human consumption from the U.S.,

76 Turkey, Mexico, and Istanbul (Ayaz, Ayaz, & Erol, 2006; Flores-Munguia, Bermudez-Almada,

77 & Vazquez-Moreno, 2000; Hsieh, Woodward, & Ho, 1995; Ozpinar, Tezmen, Gokce, & Tekiner,

78 2013).

79   Processed meat products present a challenge in terms of food fraud detection, as meat

80 species in these foods may be impossible to distinguish visually and may consist of a mixture of

81 multiple species. For example, undeclared horsemeat was found in several Mexican hamburger

82 and sausage products, as well as in raw meat samples from Turkey, which declared the products

83 as beef (Ayaz et al., 2006; Flores-Munguia et al., 2000). With the recent discovery of horsemeat

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84   in ground meat products sold for human consumption in several European countries, the

85   presence of horsemeat in U.S. consumer food and pet food products is also a concern (O'Mahony,

86   2013; Stanciu, Stanciuc, Dumitrascu, Ion, & Nistor, 2013). Considering the vast network in

87   existence of global imports and exports, it is feasible that food fraud in one part of the world

88   could spread elsewhere. One area where this possibility exists is in the cattle trade, for which the

89   U.S. is the only major exporter that does not have a mandatory cattle traceability system or

90   standards in place (Schroeder & Tonsor, 2012). Even though the USDA has implemented

91   standards for animal disease traceability, the purpose of these standards is to only regulate and

92   trace livestock moving interstate when diseased animals are found (USDA, 2013). The lack of a

93   comprehensive cattle traceability system in the U.S. may increase the potential for meat species

94   substitution and mislabeling (Shackell, 2008).

95   In addition to pet food mislabeling and food fraud, pet food safety is another area of

96   concern, especially with commercialized pet foods that are specifically formulated to address

97   immunological adverse food reactions (AFR). AFR are food allergies that may occur in both

98   dogs and cats regardless of breed, sex, or age, causing chronic dermatological disorders and

99   gastrointestinal diseases (Verlinden, Hesta, Millet, & Janssens, 2006; Vogelnest & Cheng, 2013).

100   Some common food allergens in dogs and cats include meat proteins, such as beef and chicken

101   (Raditic, Remillard, & Tater, 2011; Vogelnest & Cheng, 2013). AFR is typically diagnosed by

102   an elimination diet, which limits the number of proteins in the diet and helps to identify the cause

103   of the immunological response(s); the main treatment for AFR is to eliminate the cause of the

104   reaction (Verlinden et al., 2006). Homemade diets are usually recommended, but commercial

105   novel protein diets (NPD) and hydrolyzed protein diets (HPD) are also available on the market

106   and usually contain one protein source; therefore, it is important that these pet food products are

107   correctly labeled (Ricci et al., 2013; Verlinden et al., 2006). However, studies have shown that

108   some NPD and HPD are mislabeled. In one study, undeclared mammalian and avian DNA and

109   bone fragments were found in 10 of the 12 tested dry NPD and HPD products for dogs (Ricci et

110   al., 2013). Another study found undeclared beef proteins in a dry dog food product listing

111   venison as the only meat ingredient (Raditic et al., 2011). It is highly important to ensure that

112   these pet food products on the market are safe and correctly labeled because incorrectly labeled

113   products may cause elimination diets to fail and result in undiagnosed AFR in dogs and cats

114   suffering from mild to severe chronic immunological response(s).

115   Meat species are commonly identified in foods using either DNA or protein analyses

116   (Ballin, Vogensen, & Karlsson, 2009). Protein analyses, such as immunoassays, identify species

117   through specific antigen-antibody interactions; however, they are limited to characterizing

118   processed animal proteins (PAP) (Ballin et al., 2009). These proteins are challenging to analyze

119   in certain processed foods because some proteins are specific to certain tissues and may not be

120   found in a given product. In these circumstances, DNA-based methods, such as the polymerase

121   chain reaction (PCR), are advantageous in that DNA is found in practically all tissues and is

122   stable at higher temperatures (Ballin et al., 2009). The specific animal tissues contained in

123   processed foods are sometimes unknown and are present in mixtures; therefore, DNA analyses

124   are ideal in identifying meat species in highly processed foods (Ballin et al., 2009). Among DNA

125   targets, mitochondrial DNA (mtDNA) is desirable in these food types because it is present at a

126   higher copy number than chromosomal DNA and is therefore more likely to be detected during

127   PCR (Ballin et al., 2009). One method that shows considerable promise for identification of meat

128   species in heavily processed foods and feeds is real-time PCR (Yancy et al., 2009). This method

129   is highly sensitive, rapid, and can be used to identify species in mixed products containing meat

130   from multiple species.

131   The objective of this study was to perform a market survey of commercial canine and

132   feline pet foods in order to identify the types of meat species present in these products as well as

133   any instances of pet food mislabeling. This objective was accomplished using a real-time PCR

134   assay targeting regions of mtDNA in eight different meat species.

135   **2. Materials and Methods**

136   2.1 *Sample collection and preparation*

137   A total of 52 commercial canine and feline pet food products representing a variety of

138   meat species and processing methods were collected from retail stores in Orange County,

139   California, and online stores in July and August 2013. Each pet food product was randomly

140   assigned a unique three-digit sample identification number. The product's brand name, flavor or

141   description, net weight, ingredient list, lot number, expiration date, place of origin, and purchase

142   place and date were recorded. The USDA sample preparation and extraction standard protocols

143   (Section 17.4) for the identification of animal species in meat and poultry products were used for

144   the pet food sample preparation, with a few modifications (USDA, 2005). Sterileware scoops

145   (Scienceware, Wayne, NJ) or flame-sterilized tweezers were used to aseptically remove 30.0 g of

146   dry food products or treats that were placed into 24 oz. Whirl-Pak® Stand-up bags (Nasco, Fort

147   Atkinson, WI) with 60.0 mL of sterile water. The products were incubated at room temperature

148   for 1 h and then processed in a Seward Stomacher® 400 Circulator (Seward USA, Port Saint

149   Lucie, FL) at 230 rpm for 60 s. The entire contents of wet food products were placed in 7 oz.

150   Whirl-Pak® Write-on bags (Nasco, Fort Atkinson, WI) and the bags were hand-mixed for 60 s to

151 homogenize the samples. Two ~10 mg subsamples were collected from each product for DNA

152 extraction.

153 2.2 *DNA extraction and PCR preparation*

154 The DNA extraction portion of the Extract-N-Amp Tissue PCR Kit (#XNAT2; Sigma-

155 Aldrich, St. Louis, MO) was used to extract the DNA in duplicate from each sample using half

156 the volumes suggested by the manufacturer. Aliquots of 50.0 μL of Extraction solution and 12.5

157 μL of Tissue Preparation solution were added to each tube containing a tissue subsample. A

158 reagent blank was included with each DNA extraction as a negative control, and the samples

159 were incubated at 55ºC for 10 min, and then at 95ºC for an additional 3 min. After both

160 incubations, 50.0 μL of Neutralization Solution B was added to each sample, and then the

161 samples were centrifuged at 13,000 rpm for 1 min. The supernatant was carefully removed

162 avoiding the lipid layer when present and without disturbing the pelleted debris. The extracted

163 supernatant for each sample was then used as the extracted DNA template for real-time PCR.

164 The quantity and quality of starting DNA was not determined, as DNA extracted with this

165 method is a crude extract that could not be accurately measured with a spectrophotometer

166 (Hellberg, Kawalek, Van, Shen, & Williams-Hill, 2014).

167 2.3 *Real-Time PCR*

168 All real-time PCR amplification reactions were performed with the Rotor-Gene® Q

169 (RGQ) Real-Time PCR Cycler and software (Qiagen, Germantown, MD) and contained 12.5 μL

170 of iQ™ SYBR® Green Supermix (2X) (Bio-Rad, Hercules, CA), 1.0 μL of each oligonucleotide

171 primer (forward and reverse), 8.5 μL of sterile water, and 2.0 μL of extracted DNA or control for

172 a total reaction volume of 25.0 μL. All samples were tested for the presence of eight animal

173 species (bovine, caprine, ovine, avian [chicken, goose, turkey], porcine, and equine) using

174   species-specific primers described in previous studies (Kesmen, Sahin, & Yetim, 2007; Yancy et

175   al., 2009). The final primer concentrations in each PCR reaction were 0.16 μM for bovine, 0.25

176   μM for caprine and ovine, 0.2 μM for avian, and 0.3 μM for porcine and equine. Each PCR run

177   included the reagent blank from the DNA extraction, a no-template control, and a positive

178   control DNA. For the positive control, three 10-fold serial dilutions of DNA for each meat

179   species were made using Tris-EDTA buffer, pH 8.0 (E112-100ml; BioExpress, Kaysville, UT)

180   and were included in each PCR run. Thermocycling settings for bovine, caprine, ovine, and avian

181   were carried out as described in Yancy et al. (2009) with an initial incubation at 94ºC for 2 min

182   and then 50 cycles of 94ºC for 10 s, 58.9ºC for 15 s, and 72ºC for 40 s, with a single fluorescent

183   reading taken at the end of each cycle. The porcine and equine thermocycling conditions

184   included an initial incubation at 92ºC for 2 min and then 35 cycles of 94ºC for 50 s, 55ºC for 50 s

185   (porcine) or 62ºC for 50 s (equine), and 72ºC for 60 s with a single fluorescent reading taken at

186   the end of each cycle and a final extension at 72ºC for 5 min. These conditions were taken from

187   the protocol originally described by Kesmen et al. (2007) for use with conventional PCR and

188   were only used after sensitivity testing showed the conventional and real-time PCR results to be

189   equivalent. A melt-curve analysis was completed at the end of each run for all meat species

190   tested to confirm the specificity of amplification. Both the threshold cycle (Ct) and melt-curve

191   values and threshold were set manually by comparison with positive controls. Results were

192   determined to be positive if at least one of the subsamples tested met the criteria of (1) having a

193   Ct value for the meat species being tested and (2) having a melting temperature within 0.5 ºC of

194   the average positive control melting temperatures for that run. Results were qualitative and

195   reported in terms of presence or absence of a given species. In cases where a declared species

196   was found to be absent, additional testing was carried out to address the possibility of false

197 negatives. Each of these samples was re-extracted and re-tested in duplicate. These samples were

198 also tested with positive control tissue spikes to account for possible inhibitors in the sample

199 matrix. Positive control tissue of the declared but not detected species was mixed with the pet

200 food sample at levels of 1%, 5%, and 10%. These spiked samples were then extracted using the

201 Extract-N-Amp Tissue PCR Kit and tested with real-time PCR, as described above. All spiking

202 tests were also carried out in duplicate.

203 2.4 *Statistical analyses*

204 The rate of potentially mislabeled products was statistically compared across pet food

205 categories using IBM SPSS Statistics 21 (Armonk, NY). The rate of potentially mislabeled dog

206 food products was compared to the rate of potentially mislabeled cat food products using a

207 Pearson's chi-square test, with a pre-determined 2-sided significance value of $p < 0.05$. The rate

208 of potentially mislabeled dry foods, wet foods, and treats was compared using a Fisher's exact

209 test, with a predetermined 2-sided significance value of $p < 0.017$ (0.05/3) based on the

210 Bonferroni correction for multiple tests.

211 **3. Results and Discussion**

212 3.1 *Meat species detected in pet foods*

213 Meat species were identified and analyzed in all 52 commercial canine and feline pet

214 food products and treats collected for this study (Table 1). Some of the tested meat species in this

215 study were detected in many products while other meat species were detected in few or none of

216 the products. Of the eight meat species tested, chicken was the most commonly detected meat,

217 with 51 of the products testing positive (Fig. 1). The lower costs of chicken when compared to

218 beef or pork may explain, in part, why chicken was the most common meat ingredient detected

219 in the pet foods tested (NCC, 2012). Although the wholesale and retail prices of beef, pork, and

220   chicken have increased every year since 1960, the 2012 wholesale and retail prices of chicken

221   per pound were approximately 35% and 25% lower than wholesale and retail beef prices,

222   respectively (NCC, 2012). The 2012 wholesale and retail prices for pork were between those for

223   beef and chicken (NCC, 2012). Pork was the second most common meat species detected, with

224   positive identifications for 35 products, and beef, turkey, and lamb were detected in 34, 32, and

225   26 products, respectively (Fig. 1). Goat and goose were detected sparingly in a few products

226   containing non-specific meat ingredients (e.g., animal fat, meat and bone meal, animal digest);

227   however, they were not specifically labeled as an ingredient in any of the tested pet food

228   products.

229   With the general lack of meat authentication testing and the recent food fraud and

230   horsemeat scandal in Europe, finding horsemeat in U.S. consumer food and pet food products is

231   a concern (O'Mahony, 2013; Premanandh, 2013). Due to the ability to detect low levels of

232   horsemeat in processed food products (Kesmen et al., 2007), each pet food product in this study

233   was tested for equine DNA; however, all of the tested pet food products were negative (Table 1).

234   This finding suggests that horsemeat was not incorporated nor used as a meat substitute in any of

235   the tested pet food products ($n = 52$), including in non-specific meat ingredients.

236   More than half of the pet food products tested ($n = 38$) contained one or more non-

237   specific meat ingredient(s) (Table 1). Of those products, animal or poultry fat, meat by-products,

238   meat and bone meal (MBM), animal digest, and poultry by-product meal were the most common

239   non-specific meat ingredients listed on the product labels. The pet food industry has a large

240   demand for animal by-products, and hog (porcine) and steer (bovine) by-product values have

241   increased since 2000 (Marti, D. L., Johnson, R. J., & Mathews, K. H., Jr., 2011). The value of

242   porcine by-products has increased 80.3% between 2000 and 2010, and the value of bovine by-

243   products has risen 34.8% during the same time frame (Marti et al., 2011). Because of its use in

244   pet foods and in the medical industry, and with a rising demand on exports, animal by-product

245   use has increased over the years (Marti et al., 2011). Twenty-five products (14 dry foods and 11

246   pet treats) contained "animal fat" or "poultry fat" as an ingredient (Table 2), which is defined as

247   the fatty acid product from commercially rendered, extracted mammalian or poultry animal

248   tissue, respectively (AAFCO, 2013). Chicken was the most common species detected in these

249   products (Table 2), which may be expected considering the lower wholesale and retail prices for

250   chicken compared to those for beef and pork, as discussed above. Pork was the second most

251   common meat species detected in these products and the most common mammalian meat species

252   detected in products containing "animal fat" specifically. On the other hand, goose was the least

253   common meat species and detected in only one product that listed animal fat as its ingredient.

254   The ingredients "meat by-product" or "dried meat by-product," which are the clean and

255   non-rendered parts derived from mammals that are not considered meat or meat flesh (AAFCO,

256   2013), were included in 11 of the products tested (Table 2). Nine out of the eleven products

257   containing meat by-products as an ingredient were wet pet foods, and the other two were treats.

258   The most common detected species was pork, found in five of the 11 products. Five products (4

259   dry pet foods and 1 treat) listed MBM as an ingredient, which is considered the rendered meat

260   parts and bones from mammals (Table 2) (AAFCO, 2013). All of these products contained at

261   least two mammalian meat species, while one contained all four mammalian meat species

262   (bovine, caprine, ovine, and porcine). Additionally, "animal digest," defined as the clean and un-

263   decomposed animal tissues that have been obtained through chemical and/or enzymatic

264   hydrolysis, was included as an ingredient in five of the tested pet food products, all of which

265   were dry pet foods (Table 2) (AAFCO, 2013). Beef and chicken were detected in all of these

266   products, whereas turkey and pork were detected in four of the products, lamb was detected in

267   three of the products, and one product contained caprine meat. Poultry by-product meal

268   consisting of the ground, rendered, and clean parts of poultry was listed as an ingredient in four

269   of the tested dry pet food products (Table 2) (AAFCO, 2013). Both chicken and turkey were

270   found in all products listing "poultry by-product meal" as an ingredient, while goose was not

271   detected in any product containing poultry by-product meal.

272   3.2 *Pet food mislabeling*

273   Of the 52 products tested, 31 were found to be labeled correctly, meaning that all meat

274   species included on the product label were detected in the sample, and undeclared meat species

275   were not detected (Table 1). Twenty products were considered potentially mislabeled because

276   they either (1) contained meat species that were not included on the product label and/or (2) did

277   not contain meat species that were included on the product label. Labeling of one product (P011,

278   wet cat food) listing "meat by-products" as an ingredient could not be verified because none of

279   the five tested mammalian meat species were detected in the product. It is possible that the meat

280   by-product ingredient contained other untested mammalian meat species. Another product, P016

281   (wet cat food), listed an animal species not tested in this study (i.e., venison) as an ingredient.

282   Although the presence of venison could not be verified, the product was deemed potentially

283   mislabeled based on the possible substitution of turkey and pork for beef and lamb (Table 1).

284   Of the 20 potentially mislabeled products, 13 were dog food and 7 were cat food;

285   however, this difference was not statistically significant, according to a chi-square test (2-sided

286   p-value > 0.05). In comparing wet food, dry food, and treats, the rate of potentially mislabeled

287   wet food products ($n = 12/16$) was found to be significantly higher than the rate of potentially

288   mislabeled dry food products ($n = 2/17$), according to a Fisher's exact test with the Bonferroni

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289   correction (p value < 0.017). However, there were no significant differences between the rate of

290   potentially mislabeled treats ($n = 6/18$) and the rate of potentially mislabeled wet or dry foods.

291   Overall, these results indicate a higher frequency of mislabeling in wet foods compared to dry

292   foods for the sample set analyzed in this study. Interestingly, half of the potentially mislabeled

293   wet food products ($n = 6$) included one non-specific meat ingredient, whereas only one

294   potentially mislabeled treat product listed a non-specific meat ingredient and none of the

295   potentially mislabeled dry food products listed a non-specific meat ingredient.

296   Instances where meat species were included on the product's label but were not detected

297   in the product occurred in seven of the 20 potentially mislabeled products, with bovine being the

298   most common declared but undetected meat species (Table 1). These seven samples were

299   subjected to spiking tests with positive control tissue to address the possibility of false negatives

300   due to inhibition from the sample matrix. The results of the spiking tests with each product

301   showed that the assay was able to detect tissue from pork, lamb and chicken at levels as low as

302   1% in all the sample matrices tested, and that turkey and beef could be detected at levels as low

303   as 1−5%, depending on the product. For example, among the four products with declared but

304   undetected beef, one wet cat food product (P016) and one dog treat (P035) showed a detection

305   limit for beef of 1%, whereas two wet dog food products (P002 and P004) showed a detection

306   limit for beef of 5%. In three of the four products, beef was listed as either the first or second

307   ingredient and also appeared later in the ingredient list, suggesting that detection should have

308   been possible if the species was indeed present. Taken together, these results indicate that the

309   seven products with declared but undetected species either (1) did not contain the declared meat

310   species or (2) contained the declared meat species at levels below the detection limit for this

311   assay.

312   Meat species that were not included on the product label were detected in 16 of the 20

313   potentially mislabeled products, with pork being the most common undeclared meat species

314   detected (Table 1). For example, product P019 (dry dog food) was found to contain undeclared

315   ovine, turkey, and porcine ingredients in addition to the declared chicken and bovine ingredients.

316   In another instance with a cat treat product (P045), undeclared pork was detected in addition to

317   the declared chicken ingredients. Interestingly, in three cases, one to two meat species were

318   substituted for other meat species listed on the label. These included instances of undeclared

319   pork in place of beef in a wet dog food product (P002), undeclared turkey and pork in place of

320   beef and lamb in a wet cat food product (P016), and undeclared chicken in place of beef and

321   pork in a dog treat product (P035) (Table 1). Taken together, these results indicate a possible

322   trend for the substitution of lower-cost ingredients, such as poultry meats, for higher cost

323   ingredients, such as beef and lamb (Mundi, 2014; Raditic et al., 2011), although more research

324   would be needed to verify this trend.

325   For six products, meat species emphasized in the product name and/or description on the

326   front of the product packaging was not detected in the product. This occurred in four wet pet

327   foods and two pet treats, in which three of the products were for dogs and three for cats. The

328   declared but undetected meat species were beef, lamb, pork, and turkey, with beef being the most

329   common. Including a meat species in the product name when it is not actually detectable in the

330   product itself could be considered to be misleading according to the labeling requirements set

331   forth by the AAFCO model regulations for product naming (FDA, 2010). AAFCO's "flavor rule"

332   states that a sufficient amount of the meat or substance(s) that characterizes the meat flavor must

333   be used to avoid the product from being misleading (FDA, 2010). Product P002 (wet dog food)

334   listed "beef" in its product flavor description, and included deboned beef and beef broth as its

335 first two ingredients, respectively; however, bovine DNA was not detected in this product (Table

336 1). Instead, pork was detected, indicating a possible meat substitution and a potentially

337 misleading product to consumers (Table 1). Another example was product P017 (wet cat food),

338 which listed "turkey" in its product flavor description and as its third ingredient, but turkey DNA

339 was not detected in the product. This product contained non-specific meat ingredients; however,

340 of the eight meat species tested, chicken and goat were the only meat species detected (Table 1).

341 Product P035 (dog treats) listed both "bacon and beef" in its product description and did not

342 include any non-specific meat ingredients; however, neither porcine nor bovine DNA were

343 detected in the product. Instead, chicken was the only meat species detected in product P035

344 (Table 1). These products could potentially be misleading to consumers and may pose a risk to

345 pets with AFR to certain meat proteins.

346   Similar to the findings of the current study, previous market studies have also found a

347 number of meat products to be mislabeled (Ayaz et al., 2006; Cawthorn et al., 2013; Flores-

348 Munguia et al., 2000; Hsieh et al., 1995; Ozpinar et al., 2013; Raditic et al., 2011), and pork has

349 been found to be a commonly undeclared but detected ingredient. For example, in the South

350 African study mentioned previously, 68% of processed and packaged meat products for human

351 consumption were found to contain undeclared plant and/or animal species, with pork being the

352 most common undeclared animal species (Cawthorn et al., 2013). In several processed meat

353 samples tested in Istanbul, undeclared horse, pork, and chicken meat were detected (Ozpinar et

354 al., 2013). It was also found that pork was substituted for beef, chicken was a substitute for pork-

355 based sausages, and over half (53.4%) of samples were mislabeled (Ozpinar et al., 2013). In a

356 U.S. study conducted in Florida, meat substitution was detected in 16.6% of samples, with

357 incidences of mislabeling occurring more in cooked ground meat than in raw ground meat

16

358   products (Hsieh et al., 1995). The study also found that sheep, pork, and poultry were the most

359   common undeclared meat species. Furthermore, in a study conducted in Mexico, some samples

360   of hamburger and sausage meat contained undeclared equine and porcine meat species (Flores-

361   Munguia et al., 2000). Many of the cooked or fermented sausages and ground meat products

362   collected in Turkey contained undeclared meat species, such as cooked "beef-only" samples

363   containing poultry meat and raw "beef" samples containing horse and deer meat (Ayaz et al.,

364   2006). The results of these studies combined with the current study indicate that meat species

365   substitution and adulteration occurs in processed foods intended for either human or animal

366   consumption. Some potential factors contributing to this mislabeling trend may be (1) intentional

367   substitution with cheaper alternative meat species for economic gain or (2) unintentional

368   substitution caused by accidental cross-contamination in the production chain.

369   While a seemingly high percentage of pet foods were found to be potentially mislabeled

370   in this study, the manner in which mislabeling occurred is not clear. For example, it is unknown

371   as to whether the mislabeling was intentional or accidental and at which point(s) in the

372   production chain it took place. Real-time PCR is a sensitive assay that is capable of picking up

373   on low levels of DNA in a product. For example, the real-time PCR assay developed by Yancy et

374   al. (2009) was reported to be capable of identifying species in animal feeds at levels as low as

375   0.1%. In manufacturing and processing plants that handle more than one meat species on the

376   same equipment, some animal tissue may remain and contaminate the next product during

377   processing and handling, especially in instances where the equipment is not thoroughly cleaned

378   and sanitized between product lines (Premanandh, 2013). Another possible reason for the

379   mislabeling observed is due to a lack of traceability from the farm to the final food product

380   (Shackell, 2008), which may allow for intentional or unintentional substitution of one animal

381   product for another to go unnoticed or undocumented.

382   **4. Conclusion**

383   Although there are pet food regulations in place in the United States that are enforced by

384   federal and state entities, there is still a lack of information on meat species authentication as

385   well as accidental mislabeling and intentional food fraud. To date, few studies have been

386   published on the prevalence of meat species mislabeling in pet foods. While this study suggests

387   the occurrence of pet food mislabeling on the commercial market, further studies are needed to

388   determine the extent of mislabeling and to identify points in the production chain where

389   mislabeling occurs. Future areas of work also include the expansion of the tested meat species to

390   include seafood and uncommon meat species that have been detected in mislabeled products for

391   human consumption and testing of products marketed for pets that suffer from AFR, such as

392   those that claim to contain no animal proteins, commercial novel proteins and/or hydrolyzed

393   proteins.

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481

482 **Figure Captions.**

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

484

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	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	−	−	+	+	+	+	+	−

486   [a] Potentially mislabeled.
487   [b] Meat species listed on the product label was not detected.
488   [c] Contains undeclared meat species.
489   [d] Labeling could not be confirmed.
490

491 **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	—	—

492