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Identification of Species in Ground Meat Products Sold on the U.S. Commercial Market Using DNA-Based Methods

Identification of Species in Ground Meat Products Sold on the U.S. Commercial Market using DNA-Based Methods

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Abstract

The objective of this study was to test a variety of ground meat products sold on the U.S. commercial market for the presence of potential mislabeling. Forty-eight ground meat samples were purchased from online and retail sources, including both supermarkets and specialty meat retailers. DNA was extracted from each sample in duplicate and tested using DNA barcoding of the cytochrome *c* oxidase I (COI) gene. The resulting sequences were identified at the species level using the Barcode of Life Database (BOLD). Any samples that failed DNA barcoding went through repeat extraction and sequencing, and due to the possibility of a species mixture, they were tested with real-time polymerase chain reaction (PCR) targeting beef, chicken, lamb, turkey, pork and horse. Of the 48 samples analyzed in this study, 38 were labeled correctly and 10 were found to be mislabeled. Nine of the mislabeled samples were found to contain additional meat species based on real-time PCR, and one sample was mislabeled in its entirety. Interestingly, meat samples ordered from online specialty meat distributors had a higher rate of being mislabeled (35%) compared to samples purchased from a local butcher (18%) and samples purchased at local supermarkets (5.8%). Horsemeat, which is illegal to sell on the U.S. commercial market, was detected in two of the samples acquired from online specialty meat distributors. Overall, the mislabeling detected in this study appears to be due to either intentional mixing of lower-cost meat species into higher cost products or unintentional mixing of meat species due to cross-contamination during processing.

Keywords: DNA barcoding, ground meat, species identification, mislabeling, real-time PCR

1. Introduction

Consumers rely on the accuracy of food labeling to help them make informed food choices for purchase, whether it be for religious purposes (some religions do not permit the consumption of pork), organic and fair trade options, or allergy concerns (Ballin, 2010). However, previous market studies in Mexico, Turkey, and South Africa have reported mislabeling rates of approximately 20-70% for a variety of meat products, including sausage, ground meat, meat balls, deli meats, and dried meats (Ayaz, Ayaz, & Erol, 2006; Cawthorn, Steinman, & Hoffman, 2013; D'Amato, Alechine, Cloete, Davison, & Corach, 2013; Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno, 2000; Ozpinar, Tezmen, Gokce, & Tekiner, 2013). For example, a South African study testing processed meat products found that 68% of the samples contained species that were not declared on the package labels (Cawthorn et al., 2013). Furthermore, in a meat adulteration scandal in Europe, undeclared horsemeat was found in products labeled as 100% beef (British Broadcasting Corporation [BBC] News, 2013). In this survey conducted on lasagna products advertised as containing beef, the Food Standards Agency (FSA) found that 61% of products tested contained undeclared horsemeat. Similarly, a survey in Ireland testing a number of beef burgers, ground beef products, and salami for adulteration found that 37% of the products contained undeclared horsemeat and 85% of the products contained undeclared pork (Food Safety Authority of Ireland [FSAI], 2013). Since becoming aware of these issues, Europe has become pro-active in their testing to help prevent the sale of adulterated meat products.

In the United States, adulteration and misbranding of meat products is prohibited under the United States Code (USC) Meat Inspection Act, Title 21, Chapter 12,

Subchapter I; Inspection requirements; Adulteration and Misbranding, which states that products of animals such as cattle, sheep, swine and goats that are intended for human consumption shall not be adulterated or misbranded at the time of sale, while they are being transported in commerce, or held for sale after transportation (United States Code [USC], 2011). The United States Department of Agriculture (USDA) also monitors game meats that are domestically produced for sale in the United States (The United States Department of Agriculture [USDA], 2011), while the U.S. Food and Drug Administration (FDA) regulates imported game meats according to the Federal Food Drug and Cosmetic Act (FD&C), Chapter VIII, Section 381(m) (U.S. Food and Drug Administration [FDA], 2010). As stated in the Code of Federal Regulations (CFR) Title 9, Chapter III, Subchapter A, Part 301.2, misbranding of meat includes the use of a label that is false or misleading in any way or offering a meat product for sale under the name of another food (Code of Federal Regulations [CFR], 2014). Although there are government regulations in place, a study conducted over two decades ago in Florida, USA, reported the occurrence of meat adulteration in ground meat products, with 16.6% of the products tested found to be mislabeled (Hsieh, Woodward, & Ho, 1995). Intact meats were also tested, but none of these products was found to be mislabeled.

The above instances of mislabeling represent cases of food fraud, which may be a result of factors such as poor traceability, accidental cross contamination resulting from improper handling, inadequate cleaning of equipment between species, or intentional fraud carried out for reasons such as economic gain (Cawthorn et al., 2013; Everstine, Spink, & Kennedy, 2013; Hsieh et al., 1995; Spink & Moyer, 2011). Assessment of proper species labeling in processed products often requires DNA or protein analysis.

93 DNA barcoding is a molecular-based system that uses a standardized genetic region to
94 identify biological specimens (Hebert, Ratnasingham, & deWaard, 2003). The DNA
95 barcode for most animal species is a ~650 base-pair (bp) region of the mitochondrial gene
96 coding for cytochrome *c* oxidase subunit 1 (COI). This method has been found to be
97 highly effective in identifying many animal species, as it shows relatively low genetic
98 divergence within species and high divergence between species (Hebert, Cywinska, Ball,
99 & deWaard, 2003). Furthermore, DNA barcoding has been successfully used to identify
100 species in a variety of food products, including meat (D'Amato et al., 2013) and seafood
101 (Hellberg & Morrissey, 2011). Despite the advantages of DNA barcoding, it currently is
102 not capable of identifying multiple species in the same product (Hellberg & Morrissey,
103 2011). In these cases, alternative methods such as real-time polymerase chain reaction
104 (PCR) or next-generation sequencing must be employed.

105 Although extensive meat species testing has been carried out in Europe in light of
106 the 2013 horsemeat scandal, there has been limited research carried out on this topic in
107 the United States, with the most recent U.S. meat survey having been published in 1995.
108 Therefore, the objective of this study was to test a variety of ground meat products sold
109 on the U.S. commercial market for the presence of potential mislabeling. In cases where
110 samples failed to be identified with DNA barcoding, real-time PCR was used as a
111 supplementary test due to the possibility of a species mixture.

112 **2. Materials and Methods**

113 2.1 *Sample collection*

114 A total of 48 fresh/frozen ground meat products representing a variety of species
115 were collected for use in this project (Fig.1). Products were purchased from 5 online

specialty meat distributors and 4 retail outlets in Orange County, CA (3 supermarkets and 1 butcher). These samples represented 15 different meat types, including products labeled as antelope (n = 1), beef (n = 9), bison (n = 5), black bear (n = 1), duck (n = 1), elk (n = 3), emu (n = 1), goat (n = 1), kangaroo (n = 2), turkey (n = 7), veal (n = 2), lamb (n = 3), chicken (n = 4), pork (n = 6) and yak (n = 2). Products were packaged either as ground meat or as ground burgers/patties. Following collection, all of the products were catalogued and stored at -80 ^{o}C. Prior to sampling, products were thawed overnight at 4 ^{o}C. For each sample, a total of 30.0 ± 2.0 g was weighed into a separate, sterile 24-oz Whirl-pak bag (Nasco, Salida, CA) and homogenized with 60.0 mL of sterile water in a Stomacher® 400 Circulator (Seward, Davie, FL) at 230 rpm for 2 min (Okuma & Hellberg, 2014). Two ~10 mg subsamples of each homogenized product were then placed into two separate 1.5 mL microcentrifuge tubes for DNA extraction.

2.2 *DNA extraction*

DNA extraction was carried out in duplicate for all ground meat samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), Spin-Column protocol, with modifications described in Handy, Deeds, Ivanova, Hebert, Hanner, Ormos and Yancy (2011). Following sample collection as described above, the tissue samples were lysed with 50 µL Buffer ATL and 5.56 µL Proteinase K over a period of 1-3 h at 56 ºC with vortexing at 30 min increments. Next, 55.6 µL Buffer AL and 55.6 µL 95% ethanol were added to each sample tube and the tube was vortexed. The samples were then transferred to columns and centrifuged for 1 min at 8,000 rpm. The column membrane was washed with 140 µL of AW1 buffer and centrifuged for 1 min at 8,000 rpm followed by a second wash with 140 µL of AW2 buffer and centrifuged for 3 min at 14,000 rpm. The columns

were transferred to a sterile 1.5 mL microcentrifuge tube prior to adding 50 µL of AE buffer preheated to 37 ºC. The samples were then centrifuged for 1 min at 8,000 rpm to collect the eluted DNA. A reagent blank with no tissue added was included alongside each set of extracted samples.

2.3 *PCR and sequencing*

The mammalian primer cocktails described by Ivanova, Clare and Borisenko (2012) were used to amplify a 658-bp region of the gene coding for COI. PCR was carried out as described in Ivanova et al. (2012) except that OmniMix HS (Cepheid, Sunnyvale, CA) lyophilized PCR reagent beads were used in place of adding individual reagents and the total reaction volume was increased to 25 µL. Each reaction included the following components: 0.5 OmniMix HS PCR bead, 22.5 µL molecular grade water, 0.25 µL of each 10 µM primer cocktail, and 2 µL of DNA. Cycling conditions were followed according to Ivanova et al. (2012): 94 ºC for 2 min; 5 cycles of 94 ºC for 30 s, 50 ºC for 40 s, and 72 ºC for 1 min; 35 cycles of 94 ºC for 30 s, 55 ºC for 40 s, and 72 ºC for 1 min; and a final extension step at 72 ºC for 10 min. Thermocycling was carried out with a Mastercycler nexus gradient thermal cycler (Eppendorf, Hauppauge, NY). A non-template control (NTC) containing sterile water in place of DNA was included with each PCR run.

Confirmation of PCR was achieved as described in Hellberg, Kawalek, Van, Shen and Williams-Hill (2014) with slight modifications. PCR products (4 µL) were loaded along with sterile water (16 µL) onto pre-cast 2.0% E-gels (Life Technologies, Carlsbad, CA) and run for 6-10 min using an E-Gel iBase Power System (Life Technologies). Results were captured using Foto/Analyst Express (Fotodyne, Hartland,

WI) combined with Transilluminator FBDLT-88 (Fisher Scientific, Waltham, MA) and visualized with PCIMAGE (version 5.0.0.0 Fotodyne, Hartland, WI). Amplified products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The samples were then sent to GenScript (Piscataway, NJ) for bi-directional sequencing using BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and a 3730xl Genetic Analyzer (Life Technologies).

2.4 *Sequence analysis*

Raw sequence files were assembled and edited using Geneious R7 (Biomatters Ltd., Auckland, New Zealand). The resulting consensus sequences were then aligned using ClustalW and trimmed to the 658-bp COI DNA barcode region. The consensus sequence lengths, % high quality bases (HQ%), and number of ambiguities were recorded. Samples were considered to have been successfully sequenced if they met the following requirements outlined in Handy et al. (2011): bidirectional sequences ≥ 500 bp in length with < 2% ambiguities or a single-read ≥ 500 bp in length with ≥ 98% HQ. Consensus sequences were queried against the Barcode of Life Database (BOLD) species identification tool (http://www.boldsystems.org/) using the Species Level Barcode Records option, to determine the top species match. If a species was unable to be identified using BOLD, a search was conducted in GenBank using the Basic Local Alignment Search Tool (BLAST). The top species matches in GenBank, along with Query Coverage (%) and % Identity were recorded. Preferred common names for the identified species were determined using the Encyclopedia of Life [(EOL) (http://eol.org/)]. Any samples that failed sequencing or were initially identified as mislabeled underwent repeat DNA extraction, PCR, and sequencing. Samples that

initially failed sequencing were also tested with real-time PCR, as described below, due to the possibility of a species mixture.

2.5 *Real-time PCR*

Real-time PCR was used to test for the presence of commonly found species in ground meats (i.e., beef, lamb, chicken, turkey, and pork) as well as horse, as described in Okuma and Hellberg (2014). Amplification was carried out using a Rotor-Gene® Q Cycler (Qiagen, Germantown, MD) and each reaction tube included 12.5 µL iQ™ SYBR® Green Supermix (2X) (Bio-Rad, Hercules, CA), 8.5 µL molecular grade water, 1.0 µL of each oligonucleotide forward and reverse primer, and 2.0 µL DNA. The final primer concentrations were 0.16 µM for beef, 0.25 µM for lamb, 0.2 µM for chicken and turkey, and 0.3 µM for pork and horse. Positive DNA controls for each meat species were prepared in three 10-fold serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) using Tris-EDTA buffer, pH 8.0 (BioExpress, Kaysville, UT) and were included in each PCR run. An NTC containing sterile water in place of DNA was also run along with every set of samples. PCR cycling conditions for identification of beef, lamb, chicken, and turkey were: 94 ºC for 2 min, followed by 50 cycles of 94 ºC for 10 s, 58.9 ºC for 15 s, and 72 ºC for 40 s. Pork and horse settings were: 94 ºC for 2 min; 35 cycles of 94 ºC for 50 s, 55 ºC for 50 s, and 72 ºC for 1 min; then 72 ºC for 5 min. Melt curve analysis was completed at the end of each run. Results were determined to be positive if at least one of the subsamples tested had a Ct value for the meat species being tested and had a melting temperature within 0.5 ^{o}C of the average positive control melting temperatures for that run (Okuma & Hellberg, 2014). Results were qualitative and reported as presence or absence of the target species.

3. Results and Discussion

3.1 *DNA barcoding results*

Of the 48 samples collected in this study, 39 samples were successfully bi-directionally sequenced to assemble a COI barcode for both replicates prepared during DNA extraction (Table 1). The average sequence length for these samples was 651 ± 19 bp, the average ambiguity was 0.14 ± 0.54% and the average HQ% was 87.5 ± 12.0%. A total of 9 samples showed sequencing failure in one or both replicates. These samples underwent repeat DNA extraction and sequencing, as well as testing with real-time PCR in case of a species mixture. This follow-up testing resulted in successful sequencing for two replicates in 7 of the samples and successful sequencing for only one replicate in 2 of the samples. Based on the combination of sequencing and real-time PCR results, all 9 samples were found to contain multiple species. These samples are discussed in detail in the following section.

Among the 39 samples found to contain just one species, sequence queries against BOLD allowed for positive identification at the species level for 38 of the samples with pairwise similarities of ≥ 99.7% (Table 1). One of the samples labeled as kangaroo burgers could not be identified using BOLD and was instead queried against GenBank, which resulted in a 100% genetic match to Western grey kangaroo (*Macropus fuliginosus*). All of these samples were found to be correctly labeled except one product purchased from an online specialty meat distributor which was labeled as yak burgers but identified as cattle (*Bos taurus*)/zebu cattle (*Bos indicus*). This identification was confirmed following repeat DNA extraction and sequencing. This distributor sells ground beef products for US $22.00/kg compared to their yak burgers which retail for US

231 $43.98/kg. This is a case where economic gain is a likely cause of mislabeling, as
232 substituting the lower-cost beef for yak can result in a two-fold profit for the company.
233 Among the correctly labeled samples, 13 were purchased from online specialty meat
234 distributors, 9 were purchased from a local butcher, and 16 were purchased from local
235 supermarkets.
236 3.2 *Mixed-species samples*
237 As mentioned above, 9 of the samples tested in this study were found to contain
238 multiple species (Table 2). These samples were tested with both DNA barcoding and
239 real-time PCR, and consisted of products labeled as turkey (n = 3), lamb (n = 1), black
240 bear (n = 1), chicken (n = 1), bison (n = 1), kangaroo (n = 1) and yak (n = 1). Two of the
241 three samples labeled as ground turkey (K21 and K23) were purchased from a local
242 butcher and one sample labeled as turkey burgers (K34) was purchased from an online
243 specialty meat distributor. All three samples listed USA as country of origin. Results
244 from DNA barcoding indicated a species identity match of 100% to wild turkey
245 (*Meleagris gallopavo*) for the successful sequencing replicates originating from the two
246 samples from the local butcher, while the sample from the online specialty meat
247 distributor had one sequencing replicate with a 100% match to wild turkey and another
248 replicate with a 100% match to chicken/red junglefowl (*Gallus gallus*). Additional
249 testing with real-time PCR revealed multiple undeclared species in these products. In
250 addition to confirming the presence of turkey in all three products, real-time PCR results
251 for the turkey samples from the local butcher (K21 and K23) revealed the presence of
252 lamb, chicken, and beef, while the sample from the online specialty meat distributor
253 (K34) was positive for lamb and chicken. The undeclared species that were detected in

254 the turkey samples with real-time PCR were either more expensive than turkey (beef and
255 lamb) or considered about the same relative cost (chicken) as turkey, indicating that
256 economic fraud was not the cause of mislabeling (USDA, 2014a, 2014b). Both the local
257 butcher and the online specialty meat distributor sell several varieties of ground meats,
258 including beef, chicken and lamb. The presence of multiple species commonly found in
259 ground meats, and the fact that these retailers sell the species detected suggests the
260 possibility of cross-contamination at the processing facility. Unintentional mislabeling
261 may occur when several species are ground on the same manufacturing equipment,
262 without proper cleaning in between samples (Hsieh et al., 1995).
263 The product labeled as ground chicken (K27) that was found to contain multiple
264 species was purchased from a local supermarket and listed USA as the country of origin.
265 This sample was identified as chicken in BOLD with a 100% species identity match.
266 However, real-time PCR indicated the presence of beef, turkey and lamb in addition to
267 chicken. Because the cost of the undeclared species is typically higher than or similar to
268 the cost of chicken (USDA, 2014a, 2014b), economic gain is not suspected here and,
269 similar to the mislabeled turkey products discussed above, the mislabeling is more likely
270 due to cross-contamination at the processing facility. Importantly, the presence of
271 mammalian species in products labeled as only containing poultry is concerning for
272 individuals that are intentionally avoiding these species due to a meat allergy (Restani,
273 Ballabio, Tripodi, & Fiocchi, 2009). While meat allergies are uncommon, they can have
274 serious health consequences, such as hives, asthma or even anaphylactic shock (Restani
275 et al., 2009).

The sample labeled as yak burgers (K31) that was found to contain multiple species was purchased from an online specialty meat distributor and listed USA as the country of origin. The sequencing results for this sample initially showed a top species match to cattle with 100% genetic similarity; however, following repeat DNA extraction and sequencing, the top species match was to guanaco (*Lama guanicoe*) with 100% similarity, with secondary species matches of 99.2-99.4% to llama (*Lama glama*) and alpaca (*Lama pacos*). Guanaco, llama, and alpaca likely cannot be differentiated using the COI barcode region due to a history of interbreeding and domestication (Barreta et al., 2013). Real-time PCR results confirmed the presence of beef in the sample, with no additional species detected. The use of guanaco/llama/alpaca does not represent a case of economic gain, as the cost of ground llama and ground alpaca sold from this online specialty meat distributor (US $21.89/kg) is greater than the cost of ground yak (US $19.69/kg) sold by the same distributor. However, the use of beef in the product would be an instance of economic fraud, as the average price per kilogram for ground beef (US $9.14/kg) (USDA, 2014a) is about half that of ground yak.

The mixed-species sample labeled as black bear burgers (K30) was purchased from an online specialty meat distributor and listed USA as the country of origin. Sequencing results identified the sample as American beaver (*Castor canadensis*) with a 100% species match. Additional testing with real-time PCR on this product revealed the presence of pork in the sample as well. Interestingly, black bear burgers were previously implicated in a case of labeling fraud uncovered by the FDA (FDA, 2011). In 2011, the FDA issued a warning letter to an online specialty meat distributor on multiple accounts of food fraud stating that the black bear (*Ursus americanus*) burgers being sold were

299 found to contain elk/red deer (*Cervus* sp.) and that products labeled as black bear steaks
300 were, in actuality, brown bear (*Ursus arctos*). Similarly, the black bear burgers tested in
301 the current study were not labeled properly and represent a case of food fraud. Since the
302 cost of ground beaver offered by the same online specialty meat distributor was
303 equivalent to the cost of ground black bear, this may represent a case of substitution due
304 to mishandling or supply shortages. Alternatively, the presence of pork in the product
305 does indicate economic fraud by mixing in a lower-cost meat. This online specialty meat
306 distributor sells both black bear burgers and ground beaver meat for US $21.89/kg,
307 whereas the average cost of pork is listed at US $9.13/kg (USDA, 2014a), suggesting that
308 substitution for economic gain is a viable explanation.
309 The mixed-species sample labeled as ground kangaroo (K38) was also obtained
310 through an online specialty meat distributor and listed a country of origin of Australia.
311 This sample could not be identified at the species level in BOLD, but showed a top match
312 to Western grey kangaroo when searched in GenBank, with a genetic similarity of 96%.
313 Real-time PCR results also indicated the presence of beef in the sample. The mixing of
314 beef with kangaroo meat could be economically motivated or could be due to cross-
315 contamination during processing. This online specialty meat distributor sells ground
316 kangaroo for US $19.76/kg compared with ground beef at US $9.90/kg, resulting in a
317 potential profit to be made by mixing in the lower-cost beef with the more expensive
318 kangaroo meat.
319 Two of the samples with multiple species detected were found to contain
320 horsemeat (Table 2). These samples were labeled as ground bison (K35) and ground
321 lamb meat (K29) and were purchased from two different online specialty meat

distributors. The sample labeled as ground bison had a top match in BOLD to American elk (*Cervus canadensis*) with 97.8% genetic similarity, and real-time PCR also revealed the presence of beef, pork, and horse. The sample labeled as ground lamb was identified as lamb/sheep (*Ovis aries*) in BOLD with 100% genetic similarity and real-time PCR revealed the presence of pork and horse in addition to lamb. The sample labeled as lamb listed the USA as its country of origin, whereas the sample labeled as bison listed Canada as its country of origin. In addition to being mislabeled, these two samples are also in violation of U.S. regulations against the sale of horsemeat. In 2007, nine years after U.S. voters first passed Proposition 6, which banned the slaughter of horses and similar equines for sale for their meat for human consumption, Congress passed the American Horse Slaughter Prevention Act, prohibiting the sale of equines including horses and mules for human consumption under the Federal Meat Inspection Act (FMIA) (Library of Congress, 2011; Potter, 2012). This includes the prohibition of shipping, transporting, moving, delivering, receiving, possessing, purchasing, selling or donation of horses and other equines for human consumption (Library of Congress, 2011). Along with a nationwide ban on selling horsemeat for human consumption, some states (including California) have a law of repugnance which prevents selling any part of a horse for human consumption (California Penal Code [CPC], 1998; Roth, 2007).

Overall, mislabeling was found to be most common in products purchased from online specialty meat distributors, which showed a 35% rate of mislabeling and included products labeled as black bear and yak burgers. The next-highest rate of mislabeling (18%) was found in samples purchased from a local butcher, for which two samples labeled as ground turkey were identified as mislabeled. Local supermarkets showed the

lowest rate of mislabeling (5.8%), with just one product labeled as ground chicken found to be mislabeled.

3.3 *Comparison to previous studies*

The rate of mislabeling found in the current study of 21% is slightly higher than that found by a previous U.S. study, which reported a mislabeling rate of 16.6% for ground meats (Hsieh et al., 1995). A possible reason for the difference in these rates is that Hsieh et al. (1995) did not examine game meats, which showed a higher rate of mislabeling in the current study (27.8%) compared to the mislabeling rate for non-game meats (16.7%). Interestingly, the previous study reported that products labeled as ground beef and veal were most likely to be mislabeled or contain undeclared species, whereas in the current study, none of the products labeled as beef or veal were found to be mislabeled. However, in both studies beef was found to be a commonly undeclared species detected in products. In this study, of the 9 mislabeled samples containing mixed species, 6 were found to contain beef. Besides beef, common undeclared species found in both studies were lamb, poultry and pork. Similar to the current study, previous studies have also reported the presence of horse as an undeclared ingredient (Ayaz et al., 2006; Flores-Munguia et al., 2000). For example, a study conducted in Mexico reported horse in 39% of hamburger samples labeled as containing 100% beef (Flores-Munguia et al., 2000). The authors noted that in Mexico, horse is of lower quality and value than beef and it is regulated less than other meat species, providing the potential for it to be mixed into higher-priced ground meats. Studies conducted in South Africa have also reported widespread mislabeling of ground meats, with products containing undeclared pork and lamb, as well as high rates of mislabeling of game meats (D'Amato et al., 2013).

Similar to the current study, previous instances of mislabeling have been attributed to factors such as economic incentive, human error, improper identification and labeling of game meat species, and insufficient cleaning techniques of equipment that multiple species are ground on.

4. Conclusions

The overall results of this study indicate the presence of mislabeling in ground meat products sold on the U.S. commercial market. The majority of mislabeled products, including two samples found to contain horsemeat, were acquired from online specialty meat distributors, with only one mislabeled sample acquired from a supermarket. Despite government regulations in place to prevent misbranding of food products, it is apparent that some ground meat products are mislabeled and, in some cases, contain multiple species. The overall trends for mislabeling found in this study indicate the possibility of lower-cost species being intentionally mixed in with higher-cost species for economic gain as well as unintentional mixing of multiple species due to cross-contamination in the processing facility. The results of this study indicate the importance of continuous monitoring of commercial ground meat products for mislabeling, especially in the case of online specialty meat distributors.

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491

492 **Figure caption**

493 **Figure 1**: Summary of meat types purchased for this study, separated by retail source

494

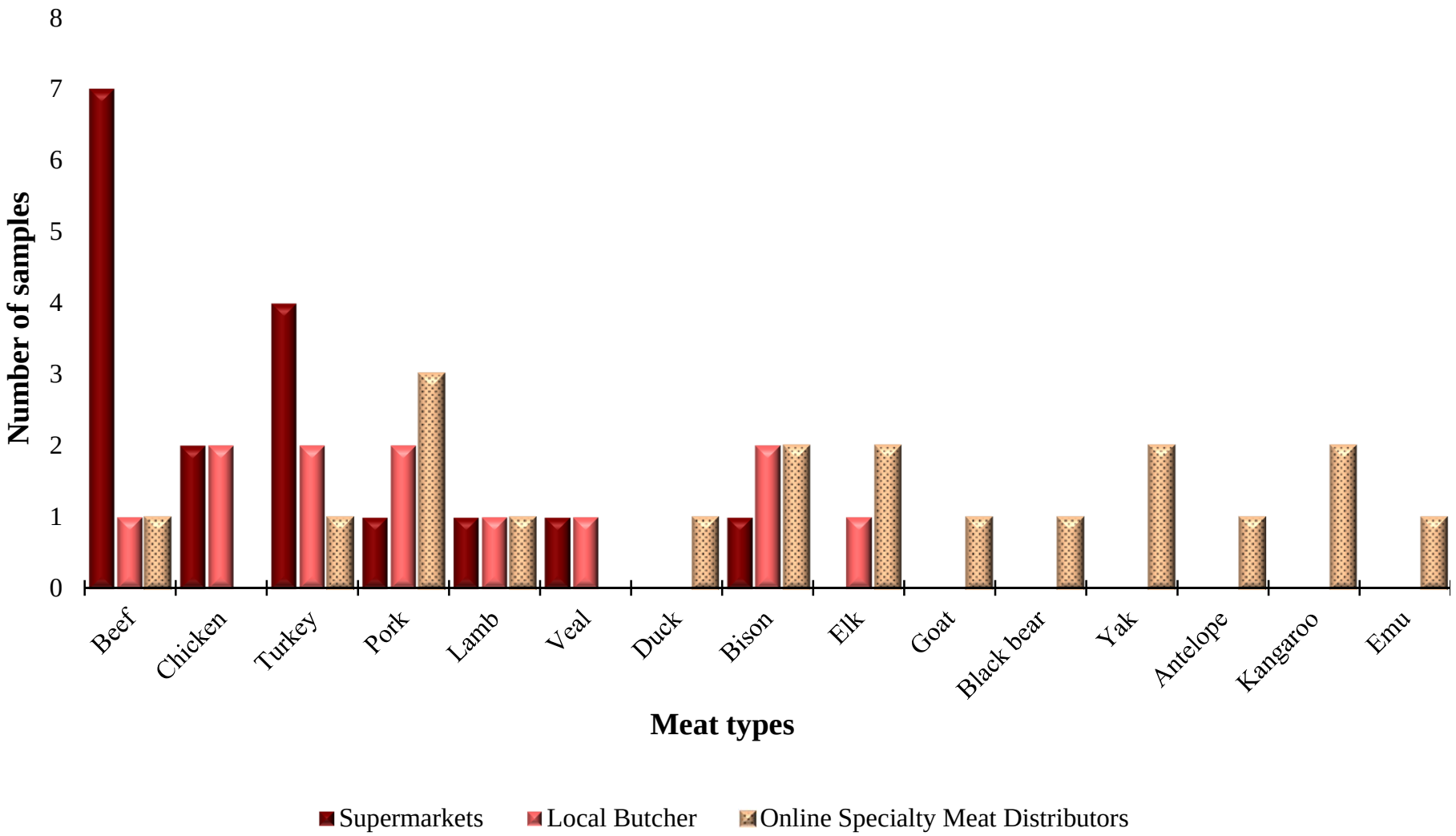
Table 1: DNA barcoding results for samples found to contain one species. Species were identified using the Barcode of Life Database (BOLD), except where otherwise noted.

Product label	Samples (n)	Genetic similarity	Top species match
Antelope	1	99.7%	Nilgai (*Boselaphus tragocamelus*)
Beef	9	100.0%	Cattle (*Bos taurus*)
Bison/Buffalo	4	99.9-100.0%	American bison (*Bison bison*)
Chicken	3	99.8-100.0%	Chicken/Red junglefowl (*Gallus gallus*)
Duck	1	100.0%	Mallard (*Anas platyrhynchos*)
Elk	3	99.8-100.0%	Red deer (*Cervus elaphus*)
Emu	1	99.8%	Emu (*Dromaius novaehollandiae*)
Goat	1	100.0%	Domestic goat (*Capra hircus*)
Kangaroo	1	100.0%[a]	Western grey kangaroo (*Macropus fuliginosus*)
Lamb	2	100.0%	Domestic sheep (*Ovis aries*)/mouflon (*O. aries musimon*)
Pork	3	99.8-100.0%	Wild boar (*Sus scrofa*)[b]
Turkey	4	99.9-100.0%	Wild turkey (*Meleagris gallopavo*)
Veal	2	100.0%	Cattle (*B. taurus*)
Wild boar	3	99.8-100.0%	Wild boar (*S. scrofa*)
Yak [c]	1	99.9-100.0%	Cattle *(B. taurus*)/Zebu cattle (*Bos indicus*)

[a] The sample sequences were not available in BOLD and were instead identified using BLAST. The % identity from GenBank is given
[b] Domestic pig (*Sus scrofa domesticus*) is a subspecies of wild boar
[c] Sample identified as mislabeled.

Table 2: Combination of DNA barcoding and real-time PCR results for samples found to contain multiple species.

Sample number	Product label	Top species match with DNA barcoding	Genetic similarity	Real-time PCR results					
				Beef	**Pork**	**Turkey**	**Sheep/Lamb**	**Chicken**	**Horse**
K30	Black bear	American beaver (*Castor canadensis*)	100.0%	—	+	—	—	—	—
K35	Bison	American elk (*Cervus canadensis*)	97.8%	+	+	—	—	—	+
K27	Chicken	Chicken/Red junglefowl (*Gallus gallus*)	100.0%	+	—	+	+	+	—
K38	Kangaroo	Western grey kangaroo (*Macropus fuliginosus*)	96%	+	—	—	—	—	—
K29	Lamb	Domestic sheep (*Ovis aries)*/Mouflon (*O. aries musimon*)	100.0%	—	+	—	+	—	+
K21	Turkey	Wild turkey (*Meleagris gallopavo*)	100.0%	+	—	+	+	+	—
K23	Turkey	Wild turkey (*M.gallopavo*)	100.0%	+	—	+	+	+	—
K34	Turkey	Wild turkey (*M.gallopavo*); Chicken (*G. gallus*)	100.0%; 100.0%	—	—	+	+	+	—
K31	Yak	Guanaco (*Lama guanicoe*); Cattle (*Bos taurus*)	100.0%; 100.0%	+	—	—	—	—	—


8
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4
3
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1
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Number of samples
Beef
Chicken
Turkey
Pork
Lamb
Veal
Duck
Bison
Elk
Goat
Black bear
Yak
Antelope
Kangaroo
Emu
Meat types
Supermarkets
Local Butcher
Online Specialty Meat Distributors