

1-2016

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


Dawn Kane

Chapman University, dawnkane@cox.net

Rosalee S. Hellberg

Chapman University, hellberg@chapman.edu

Follow this and additional works at: http://digitalcommons.chapman.edu/food_science_articles

 Part of the [Food Processing Commons](#), [Meat Science Commons](#), [Other Food Science Commons](#), and the [Poultry or Avian Science Commons](#)

Recommended Citation

Kane, D.E., Hellberg, R.S., 2016. Identification of species in ground meat products sold on the U.S. commercial market using DNA-based methods. *Food Control* 59, 158–163. doi:10.1016/j.foodcont.2015.05.020

This Article is brought to you for free and open access by the Food Science at Chapman University Digital Commons. It has been accepted for inclusion in Food Science Faculty Articles and Research by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.

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

Comments

NOTICE: this is the author's version of a work that was accepted for publication in *Food Control*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Food Control*, volume 59, in 2016. DOI: [10.1016/j.foodcont.2015.05.020](https://doi.org/10.1016/j.foodcont.2015.05.020)

The Creative Commons license below applies only to this version of the article.

Creative Commons License



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 4.0 License](https://creativecommons.org/licenses/by-nc-nd/4.0/).

Copyright

Elsevier

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

3 Dawn E. Kane and Rosalee S. Hellberg*

4 Chapman University, Schmid College of Science and Technology, Food Science and
5 Nutrition, One University Drive, Orange, CA 92866

6

7

8 ***Corresponding Author**

9 Rosalee S. Hellberg, Ph.D.

10 Chapman University

11 Phone: (714) 628-2811

12 Fax: (714) 289-2041

13 E-mail: hellberg@chapman.edu

14

15

16

17

18

19

20

21

22

23

24 **Abstract**

25 The objective of this study was to test a variety of ground meat products sold on
26 the U.S. commercial market for the presence of potential mislabeling. Forty-eight ground
27 meat samples were purchased from online and retail sources, including both supermarkets
28 and specialty meat retailers. DNA was extracted from each sample in duplicate and
29 tested using DNA barcoding of the cytochrome *c* oxidase I (COI) gene. The resulting
30 sequences were identified at the species level using the Barcode of Life Database
31 (BOLD). Any samples that failed DNA barcoding went through repeat extraction and
32 sequencing, and due to the possibility of a species mixture, they were tested with real-
33 time polymerase chain reaction (PCR) targeting beef, chicken, lamb, turkey, pork and
34 horse. Of the 48 samples analyzed in this study, 38 were labeled correctly and 10 were
35 found to be mislabeled. Nine of the mislabeled samples were found to contain additional
36 meat species based on real-time PCR, and one sample was mislabeled in its entirety.
37 Interestingly, meat samples ordered from online specialty meat distributors had a higher
38 rate of being mislabeled (35%) compared to samples purchased from a local butcher
39 (18%) and samples purchased at local supermarkets (5.8%). Horsemeat, which is illegal
40 to sell on the U.S. commercial market, was detected in two of the samples acquired from
41 online specialty meat distributors. Overall, the mislabeling detected in this study appears
42 to be due to either intentional mixing of lower-cost meat species into higher cost products
43 or unintentional mixing of meat species due to cross-contamination during processing.

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

46

47 **1. Introduction**

48 Consumers rely on the accuracy of food labeling to help them make informed
49 food choices for purchase, whether it be for religious purposes (some religions do not
50 permit the consumption of pork), organic and fair trade options, or allergy concerns
51 (Ballin, 2010). However, previous market studies in Mexico, Turkey, and South Africa
52 have reported mislabeling rates of approximately 20-70% for a variety of meat products,
53 including sausage, ground meat, meat balls, deli meats, and dried meats (Ayaz, Ayaz, &
54 Erol, 2006; Cawthorn, Steinman, & Hoffman, 2013; D'Amato, Alechine, Cloete,
55 Davison, & Corach, 2013; Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno,
56 2000; Ozpinar, Tezmen, Gokce, & Tekiner, 2013). For example, a South African study
57 testing processed meat products found that 68% of the samples contained species that
58 were not declared on the package labels (Cawthorn et al., 2013). Furthermore, in a meat
59 adulteration scandal in Europe, undeclared horsemeat was found in products labeled as
60 100% beef (British Broadcasting Corporation [BBC] News, 2013). In this survey
61 conducted on lasagna products advertised as containing beef, the Food Standards Agency
62 (FSA) found that 61% of products tested contained undeclared horsemeat. Similarly, a
63 survey in Ireland testing a number of beef burgers, ground beef products, and salami for
64 adulteration found that 37% of the products contained undeclared horsemeat and 85% of
65 the products contained undeclared pork (Food Safety Authority of Ireland [FSAI], 2013).
66 Since becoming aware of these issues, Europe has become pro-active in their testing to
67 help prevent the sale of adulterated meat products.

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

70 Subchapter I; Inspection requirements; Adulteration and Misbranding, which states that
71 products of animals such as cattle, sheep, swine and goats that are intended for human
72 consumption shall not be adulterated or misbranded at the time of sale, while they are
73 being transported in commerce, or held for sale after transportation (United States Code
74 [USC], 2011). The United States Department of Agriculture (USDA) also monitors game
75 meats that are domestically produced for sale in the United States (The United States
76 Department of Agriculture [USDA], 2011), while the U.S. Food and Drug Administration
77 (FDA) regulates imported game meats according to the Federal Food Drug and Cosmetic
78 Act (FD&C), Chapter VIII, Section 381(m) (U.S. Food and Drug Administration [FDA],
79 2010). As stated in the Code of Federal Regulations (CFR) Title 9, Chapter III,
80 Subchapter A, Part 301.2, misbranding of meat includes the use of a label that is false or
81 misleading in any way or offering a meat product for sale under the name of another food
82 (Code of Federal Regulations [CFR], 2014). Although there are government regulations
83 in place, a study conducted over two decades ago in Florida, USA, reported the
84 occurrence of meat adulteration in ground meat products, with 16.6% of the products
85 tested found to be mislabeled (Hsieh, Woodward, & Ho, 1995). Intact meats were also
86 tested, but none of these products was found to be mislabeled.

87 The above instances of mislabeling represent cases of food fraud, which may be a
88 result of factors such as poor traceability, accidental cross contamination resulting from
89 improper handling, inadequate cleaning of equipment between species, or intentional
90 fraud carried out for reasons such as economic gain (Cawthorn et al., 2013; Everstine,
91 Spink, & Kennedy, 2013; Hsieh et al., 1995; Spink & Moyer, 2011). Assessment of
92 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

116 specialty meat distributors and 4 retail outlets in Orange County, CA (3 supermarkets and
117 1 butcher). These samples represented 15 different meat types, including products
118 labeled as antelope (n = 1), beef (n = 9), bison (n = 5), black bear (n = 1), duck (n = 1),
119 elk (n = 3), emu (n = 1), goat (n = 1), kangaroo (n = 2), turkey (n = 7), veal (n = 2), lamb
120 (n = 3), chicken (n = 4), pork (n = 6) and yak (n = 2). Products were packaged either as
121 ground meat or as ground burgers/patties. Following collection, all of the products were
122 catalogued and stored at -80 °C. Prior to sampling, products were thawed overnight at 4
123 °C. For each sample, a total of 30.0 ± 2.0 g was weighed into a separate, sterile 24-oz
124 Whirl-pak bag (Nasco, Salida, CA) and homogenized with 60.0 mL of sterile water in a
125 Stomacher[®] 400 Circulator (Seward, Davie, FL) at 230 rpm for 2 min (Okuma &
126 Hellberg, 2014). Two ~10 mg subsamples of each homogenized product were then
127 placed into two separate 1.5 mL microcentrifuge tubes for DNA extraction.

128 *2.2 DNA extraction*

129 DNA extraction was carried out in duplicate for all ground meat samples using
130 the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), Spin-Column protocol, with
131 modifications described in Handy, Deeds, Ivanova, Hebert, Hanner, Ormos and Yancy
132 (2011). Following sample collection as described above, the tissue samples were lysed
133 with 50 μ L Buffer ATL and 5.56 μ L Proteinase K over a period of 1-3 h at 56 °C with
134 vortexing at 30 min increments. Next, 55.6 μ L Buffer AL and 55.6 μ L 95% ethanol were
135 added to each sample tube and the tube was vortexed. The samples were then transferred
136 to columns and centrifuged for 1 min at 8,000 rpm. The column membrane was washed
137 with 140 μ L of AW1 buffer and centrifuged for 1 min at 8,000 rpm followed by a second
138 wash with 140 μ L of AW2 buffer and centrifuged for 3 min at 14,000 rpm. The columns

139 were transferred to a sterile 1.5 mL microcentrifuge tube prior to adding 50 μ L of AE
140 buffer preheated to 37 $^{\circ}$ C. The samples were then centrifuged for 1 min at 8,000 rpm to
141 collect the eluted DNA. A reagent blank with no tissue added was included alongside
142 each set of extracted samples.

143 *2.3 PCR and sequencing*

144 The mammalian primer cocktails described by Ivanova, Clare and Borisenko
145 (2012) were used to amplify a 658-bp region of the gene coding for COI. PCR was
146 carried out as described in Ivanova et al. (2012) except that OmniMix HS (Cepheid,
147 Sunnyvale, CA) lyophilized PCR reagent beads were used in place of adding individual
148 reagents and the total reaction volume was increased to 25 μ L. Each reaction included
149 the following components: 0.5 OmniMix HS PCR bead, 22.5 μ L molecular grade water,
150 0.25 μ L of each 10 μ M primer cocktail, and 2 μ L of DNA. Cycling conditions were
151 followed according to Ivanova et al. (2012): 94 $^{\circ}$ C for 2 min; 5 cycles of 94 $^{\circ}$ C for 30 s,
152 50 $^{\circ}$ C for 40 s, and 72 $^{\circ}$ C for 1 min; 35 cycles of 94 $^{\circ}$ C for 30 s, 55 $^{\circ}$ C for 40 s, and 72 $^{\circ}$ C
153 for 1 min; and a final extension step at 72 $^{\circ}$ C for 10 min. Thermocycling was carried out
154 with a Mastercycler nexus gradient thermal cycler (Eppendorf, Hauppauge, NY). A non-
155 template control (NTC) containing sterile water in place of DNA was included with each
156 PCR run.

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

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

168 *2.4 Sequence analysis*

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

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

187 *2.5 Real-time PCR*

188 Real-time PCR was used to test for the presence of commonly found species in
189 ground meats (i.e., beef, lamb, chicken, turkey, and pork) as well as horse, as described in
190 Okuma and Hellberg (2014). Amplification was carried out using a Rotor-Gene[®] Q
191 Cycler (Qiagen, Germantown, MD) and each reaction tube included 12.5 μL iQ[™]
192 SYBR[®] Green Supermix (2X) (Bio-Rad, Hercules, CA), 8.5 μL molecular grade water,
193 1.0 μL of each oligonucleotide forward and reverse primer, and 2.0 μL DNA. The final
194 primer concentrations were 0.16 μM for beef, 0.25 μM for lamb, 0.2 μM for chicken and
195 turkey, and 0.3 μM for pork and horse. Positive DNA controls for each meat species
196 were prepared in three 10-fold serial dilutions (10^{-1} , 10^{-2} and 10^{-3}) using Tris-EDTA
197 buffer, pH 8.0 (BioExpress, Kaysville, UT) and were included in each PCR run. An NTC
198 containing sterile water in place of DNA was also run along with every set of samples.
199 PCR cycling conditions for identification of beef, lamb, chicken, and turkey were: 94 °C
200 for 2 min, followed by 50 cycles of 94 °C for 10 s, 58.9 °C for 15 s, and 72 °C for 40 s.
201 Pork and horse settings were: 94 °C for 2 min; 35 cycles of 94 °C for 50 s, 55 °C for 50 s,
202 and 72 °C for 1 min; then 72 °C for 5 min. Melt curve analysis was completed at the end
203 of each run. Results were determined to be positive if at least one of the subsamples
204 tested had a Ct value for the meat species being tested and had a melting temperature
205 within 0.5 °C of the average positive control melting temperatures for that run (Okuma &
206 Hellberg, 2014). Results were qualitative and reported as presence or absence of the
207 target species.

208 **3. Results and Discussion**

209 3.1 *DNA barcoding results*

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

221 Among the 39 samples found to contain just one species, sequence queries against
222 BOLD allowed for positive identification at the species level for 38 of the samples with
223 pairwise similarities of $\geq 99.7\%$ (Table 1). One of the samples labeled as kangaroo
224 burgers could not be identified using BOLD and was instead queried against GenBank,
225 which resulted in a 100% genetic match to Western grey kangaroo (*Macropus*
226 *fuliginosus*). All of these samples were found to be correctly labeled except one product
227 purchased from an online specialty meat distributor which was labeled as yak burgers but
228 identified as cattle (*Bos taurus*)/zebu cattle (*Bos indicus*). This identification was
229 confirmed following repeat DNA extraction and sequencing. This distributor sells
230 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).

276 The sample labeled as yak burgers (K31) that was found to contain multiple
277 species was purchased from an online specialty meat distributor and listed USA as the
278 country of origin. The sequencing results for this sample initially showed a top species
279 match to cattle with 100% genetic similarity; however, following repeat DNA extraction
280 and sequencing, the top species match was to guanaco (*Lama guanicoe*) with 100%
281 similarity, with secondary species matches of 99.2-99.4% to llama (*Lama glama*) and
282 alpaca (*Lama pacos*). Guanaco, llama, and alpaca likely cannot be differentiated using
283 the COI barcode region due to a history of interbreeding and domestication (Barreta et
284 al., 2013). Real-time PCR results confirmed the presence of beef in the sample, with no
285 additional species detected. The use of guanaco/llama/alpaca does not represent a case of
286 economic gain, as the cost of ground llama and ground alpaca sold from this online
287 specialty meat distributor (US \$21.89/kg) is greater than the cost of ground yak (US
288 \$19.69/kg) sold by the same distributor. However, the use of beef in the product would
289 be an instance of economic fraud, as the average price per kilogram for ground beef (US
290 \$9.14/kg) (USDA, 2014a) is about half that of ground yak.

291 The mixed-species sample labeled as black bear burgers (K30) was purchased
292 from an online specialty meat distributor and listed USA as the country of origin.
293 Sequencing results identified the sample as American beaver (*Castor canadensis*) with a
294 100% species match. Additional testing with real-time PCR on this product revealed the
295 presence of pork in the sample as well. Interestingly, black bear burgers were previously
296 implicated in a case of labeling fraud uncovered by the FDA (FDA, 2011). In 2011, the
297 FDA issued a warning letter to an online specialty meat distributor on multiple accounts
298 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

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

340 Overall, mislabeling was found to be most common in products purchased from
341 online specialty meat distributors, which showed a 35% rate of mislabeling and included
342 products labeled as black bear and yak burgers. The next-highest rate of mislabeling
343 (18%) was found in samples purchased from a local butcher, for which two samples
344 labeled as ground turkey were identified as mislabeled. Local supermarkets showed the

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

347 *3.3 Comparison to previous studies*

348 The rate of mislabeling found in the current study of 21% is slightly higher than
349 that found by a previous U.S. study, which reported a mislabeling rate of 16.6% for
350 ground meats (Hsieh et al., 1995). A possible reason for the difference in these rates is
351 that Hsieh et al. (1995) did not examine game meats, which showed a higher rate of
352 mislabeling in the current study (27.8%) compared to the mislabeling rate for non-game
353 meats (16.7%). Interestingly, the previous study reported that products labeled as ground
354 beef and veal were most likely to be mislabeled or contain undeclared species, whereas in
355 the current study, none of the products labeled as beef or veal were found to be
356 mislabeled. However, in both studies beef was found to be a commonly undeclared
357 species detected in products. In this study, of the 9 mislabeled samples containing mixed
358 species, 6 were found to contain beef. Besides beef, common undeclared species found
359 in both studies were lamb, poultry and pork. Similar to the current study, previous
360 studies have also reported the presence of horse as an undeclared ingredient (Ayaz et al.,
361 2006; Flores-Munguia et al., 2000). For example, a study conducted in Mexico reported
362 horse in 39% of hamburger samples labeled as containing 100% beef (Flores-Munguia et
363 al., 2000). The authors noted that in Mexico, horse is of lower quality and value than
364 beef and it is regulated less than other meat species, providing the potential for it to be
365 mixed into higher-priced ground meats. Studies conducted in South Africa have also
366 reported widespread mislabeling of ground meats, with products containing undeclared
367 pork and lamb, as well as high rates of mislabeling of game meats (D'Amato et al., 2013).

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

372 **4. Conclusions**

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

385 **Acknowledgements**

386 The authors would like to thank Chapman University Schmid College of Science and
387 Technology, the Office of the Chancellor and the Graduate Academic Council for grant
388 support. None of these entities were involved with the design or execution of the study.

389 **References**

390 Ayaz, Y., Ayaz, N. D., & Erol, I. (2006). Detection of species in meat and meat products
391 using enzyme-linked immunosorbent assay. *J. Muscle Foods*, 17(2), 214-220.
392 <http://dx.doi.org/10.1111/j.1745-4573.2006.00046.x>

393 Ballin, N. Z. (2010). Authentication of meat and meat products. *Meat Sci.*, 86(3), 577-
394 587. <http://dx.doi.org/10.1016/j.meatsci.2010.06.001>

395 Barreta, J., Gutierrez-Gil, B., Iniguez, V., Saavedra, V., Chiri, R., Latorre, E., et al.,
396 (2013). Analysis of mitochondrial DNA in Bolivian llama, alpaca and vicuna
397 populations: A contribution to the phylogeny of the South American camelids.
398 *Anim. Genet.*, 44(2), 158-168. [http://dx.doi.org/10.1111/j.1365-](http://dx.doi.org/10.1111/j.1365-2052.2012.02376.x)
399 [2052.2012.02376.x](http://dx.doi.org/10.1111/j.1365-2052.2012.02376.x)

400 BBC News. (2013). Findus beef lasagne contains up to 100% horsemeat, FSA says. *BBC*
401 *News UK*. Accessible at <http://www.bbc.co.uk/news/uk-21375594> Accessed
402 11.12.14

403 Cawthorn, D.-M., Steinman, H. A., & Hoffman, L. C. (2013). A high incidence of species
404 substitution and mislabelling detected in meat products sold in South Africa. *Food*
405 *Control*, 32(2), 440-449. <http://dx.doi.org/10.1016/j.foodcont.2013.01.008>

406 CFR. (2014). *Code of Federal Regulations*. Washington, DC: U.S. Gov. Print. Off.
407 Accessible at
408 [http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR&sea](http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR&searchPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+301&oldPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+303&isCollapsed=true&selectedYearFrom=2014&ycord=360)
409 [rchPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+301&oldPath=Title](http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR&searchPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+301&oldPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+303&isCollapsed=true&selectedYearFrom=2014&ycord=360)
410 [+9%2FChapter+III%2FSubchapter+A%2FPart+303&isCollapsed=true&selected](http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR&searchPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+301&oldPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+303&isCollapsed=true&selectedYearFrom=2014&ycord=360)
411 [YearFrom=2014&ycord=360](http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR&searchPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+301&oldPath=Title+9%2FChapter+III%2FSubchapter+A%2FPart+303&isCollapsed=true&selectedYearFrom=2014&ycord=360). Accessed 09.12.14

412 CPC. (1998, 2012). California Penal Code 598. Accessible at
413 [http://www.leginfo.ca.gov/cgi-bin/displaycode?section=pen&group=00001-](http://www.leginfo.ca.gov/cgi-bin/displaycode?section=pen&group=00001-01000&file=594-625c)
414 [01000&file=594-625c](http://www.leginfo.ca.gov/cgi-bin/displaycode?section=pen&group=00001-01000&file=594-625c) Accessed 08.11.14

415 D'Amato, M. E., Alechine, E., Cloete, K. W., Davison, S., & Corach, D. (2013). Where is
416 the game? Wild meat products authentication in South Africa: A case study.
417 *Invest. Genet.*, 4(1). <http://dx.doi.org/10.1186/2041-2223-4-6>

418 Everstine, K., Spink, J., & Kennedy, S. (2013). Economically motivated adulteration
419 (EMA) of food: Common characteristics of EMA incidents. *J. Food Prot.*, 76(4),
420 723-735. <http://dx.doi.org/10.4315/0362-028x.jfp-12-399>

421 FDA. (2010). Federal Food, Drug, and Cosmetic Act (FD&C Act). 2006. Accessible at
422 [http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCos-](http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCA/default.htm)
423 [meticActFDCA/default.htm](http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCA/default.htm) Accessed 20.12.14

424 FDA. (2011). *U.S. Food and Drug Administration: Warning letter 2/4/11*. Chicago, IL:
425 U.S. Food and Drug Administration Accessible at
426 [http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2011/ucm24289](http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2011/ucm242890.htm)
427 [0.htm](http://www.fda.gov/ICECI/EnforcementActions/WarningLetters/2011/ucm242890.htm). Accessed 24.12.14

428 Flores-Munguia, M. E., Bermudez-Almada, M. C., & Vazquez-Moreno, L. (2000). A
429 research note: Detection of adulteration in processed traditional meat products. *J.*
430 *Muscle Foods*, 11(4), 319-325. [http://dx.doi.org/10.1111/j.1745-](http://dx.doi.org/10.1111/j.1745-4573.2000.tb00435.x)
431 [4573.2000.tb00435.x](http://dx.doi.org/10.1111/j.1745-4573.2000.tb00435.x)

432 FSAI. (2013). FSAI survey finds horse DNA in some beef burger products. *Food Safety*
433 *Authority of Ireland*. Accessible at

434 http://www.fsai.ie/news_centre/press_releases/horseDNA15012013.html
435 Accessed 12.12.14

436 Handy, S. M., Deeds, J., R., Ivanova, N. V., Hebert, P. D. N., Hanner, R., Ormos, A.,
437 Yancy, H. F. (2011). Single laboratory validated method for DNA-barcoding for
438 the species identification of fish for FDA regulatory compliance (Vol. 2014).
439 Silver Spring, MD: U.S. Food and Drug Administration.

440 Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological
441 identifications through DNA barcodes. *P. Roy. Soc. B-Biol. Sci.*, 270(1512), 313-
442 321. <http://dx.doi.org/10.1098/rspb.2002.2218>

443 Hebert, P. D. N., Ratnasingham, S., & deWaard, J. R. (2003). Barcoding animal life:
444 Cytochrome c oxidase subunit 1 divergences among closely related species. *P.*
445 *Roy. Soc. B-Biol. Sci.*, 270, S96-S99. <http://dx.doi.org/10.1098/rsbl.2003.0025>

446 Hellberg, R. S., Kawalek, M. D., Van, K. T., Shen, Y., & Williams-Hill, D. M. (2014).
447 Comparison of DNA extraction and PCR setup methods for use in high-
448 throughput DNA barcoding of fish species. *Food Anal. Method*, 7(10).
449 <http://dx.doi.org/10.1007/s12161-014-9865-z>

450 Hellberg, R. S. R., & Morrissey, M. T. (2011). Advances in DNA-based techniques for
451 the detection of seafood species substitution on the commercial market. *JALA*,
452 16(4), 308-321. <http://dx.doi.org/10.1016/j.jala.2010.07.004>

453 Hsieh, Y.-H. P., Woodward, B. B., & Ho, S.-H. (1995). Detection of species
454 substitution in raw and cooked meats using immunoassays. *J. Food Prot.*, 58(5),
455 555-559.

456 Ivanova, N. V., Clare, E. L., & Borisenko, A. V. (2012). DNA barcoding in mammals.
457 *Methods Mol. Biol.*, 858, 153-182. [http://dx.doi.org/10.1007/978-1-61779-591-](http://dx.doi.org/10.1007/978-1-61779-591-6_8)
458 [6_8](http://dx.doi.org/10.1007/978-1-61779-591-6_8)

459 Library of Congress. (2011). American Horse Slaughter Prevention Act. *H.R.2966*,
460 *H.R.857*. Accessible at [http://www.congress.gov/bill/112th-congress/house-](http://www.congress.gov/bill/112th-congress/house-bill/2966)
461 [bill/2966](http://www.congress.gov/bill/112th-congress/house-bill/2966) Accessed 18.12.14

462 Okuma, T. A., & Hellberg, R. S. (2014). Identification of meat species in pet foods using
463 a real-time polymerase chain reaction (PCR) assay. *Food Control*, 50, 9-17.
464 <http://dx.doi.org/10.1016/j.foodcont.2014.08.017>

465 Ozpinar, H., Tezmen, G., Gokce, I., & Tekiner, I. H. (2013). Detection of animal species
466 in some meat and meat products by comparatively using DNA microarray and
467 real time PCR methods. *Kafkas Univ. Vet. Fak.*, 19(2), 245-252.
468 <http://dx.doi.org/10.9775/kvfd.2012.7616>

469 Potter, L. (2012). A timeline of horse slaughter legislation in the United States.
470 Accessible at [http://www.horsechannel.com/horse-resources/horse-slaughter-](http://www.horsechannel.com/horse-resources/horse-slaughter-timeline.aspx)
471 [timeline.aspx](http://www.horsechannel.com/horse-resources/horse-slaughter-timeline.aspx) Accessed 01.12.14

472 Restani, P., Ballabio, C., Tripodi, S., & Fiocchi, A. (2009). Meat allergy. *Curr. Opin.*
473 *Allergy. Cl.*, 9(3), 265-269. <http://dx.doi.org/10.1097/ACI.0b013e32832aef3d>

474 Roth, A. E. (2007). Repugnance as a constraint on markets. *J. Econ. Perspect.*, 21(3), 37-
475 58. <http://dx.doi.org/10.1257/jep.21.3.37>

476 Spink, J., & Moyer, D. C. (2011). Defining the public health threat of food fraud. *J. Food*
477 *Sci.*, 76(9), R157-R163. <http://dx.doi.org/10.1111/j.1750-3841.2011.02417.x>

478 USC. (2011). United States Code. Title 21. Chapter 12. Subchapter IV sec 676(a).
479 Accessible at [http://www.gpo.gov/fdsys/granule/USCODE-2010-
title21/USCODE-2010-title21-chap12-subchapIV-sec676](http://www.gpo.gov/fdsys/granule/USCODE-2010-
480 title21/USCODE-2010-title21-chap12-subchapIV-sec676) Accessed 13.11.14
481 USDA. (2011). Game from farm to table. *Food Safety Information*. Accessible at
482 [http://www.fsis.usda.gov/wps/wcm/connect/e432ba38-79f6-42c8-af50-
df7cf788a298/Game_from_Farm_to_Table.pdf?MOD=AJPERES](http://www.fsis.usda.gov/wps/wcm/connect/e432ba38-79f6-42c8-af50-
483 df7cf788a298/Game_from_Farm_to_Table.pdf?MOD=AJPERES) Accessed
484 12.12.14
485 USDA. (2014a). Economic Research Service. Accessible at
486 <http://www.ers.usda.gov/data-products/meat-price-spreads.aspx> Accessed
487 12.12.14
488 USDA. (2014b). USDA Weekly National Lamb Market Summary (Vol. 21).
489 Washington, DC: U. S. Dep. Agric. Accessible at
490 <http://www.ams.usda.gov/mnreports/lswlamb.pdf> Accessed 18.12.14

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

^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 domestica*) 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 (<i>Castor canadensis</i>)	100.0%	—	+	—	—	—	—
K35	Bison	American elk (<i>Cervus canadensis</i>)	97.8%	+	+	—	—	—	+
K27	Chicken	Chicken/Red junglefowl (<i>Gallus gallus</i>)	100.0%	+	—	+	+	+	—
K38	Kangaroo	Western grey kangaroo (<i>Macropus fuliginosus</i>)	96%	+	—	—	—	—	—
K29	Lamb	Domestic sheep (<i>Ovis aries</i>)/Mouflon (<i>O. aries musimon</i>)	100.0%	—	+	—	+	—	+
K21	Turkey	Wild turkey (<i>Meleagris gallopavo</i>)	100.0%	+	—	+	+	+	—
K23	Turkey	Wild turkey (<i>M.gallopavo</i>)	100.0%	+	—	+	+	+	—
K34	Turkey	Wild turkey (<i>M.gallopavo</i>); Chicken (<i>G. gallus</i>)	100.0%; 100.0%	—	—	+	+	+	—
K31	Yak	Guanaco (<i>Lama guanicoe</i>); Cattle (<i>Bos taurus</i>)	100.0%; 100.0%	+	—	—	—	—	—

