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Species Identification of Canned Meat Products by Using DNA-Based Methods

Alaa Tariq Abdulwahid[1], Huda Kaduim Kareem[2]

[1]Assist. Prof. Department of Veterinary Public Medicine, College of Veterinary Medicine, University of Basrah, Iraq, [2]Assist. Lecturer. Department of Biology, Collage of Education for Pure Sciences, University of Basrah, Basrah, Iraq

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Abstract

Fraud among different meats became great issue concerns of economical and hygienic aspects. Canned meat products are widely adulterated for economically motivated. The objective of the current study was to test a variety of canned meat products sold on the Iraq/Basrah commercial market to assure the authenticity of meat products for quality and safety purposes. Twenty seven canned meat samples were purchased from supermarkets. They were targeting cattle, sheep, goat, horse, donkey, and chicken for the possibility of a species mixture. DNA was extracted from each sample in triplicate and tested using DNA–based method. Samples analysed in the current study were found to be mislabeled. Meat species (chicken and beef) were detected in the samples collected from supermarkets that are not declared in their labels. Overall, the mislabeling identified in the current study either due to mixing of cheaper meat species into more expensive or mixing of meat species during processing due to cross-contamination, unintentionally.

Key words: Canned meat, fraud, identification method, DNA technology.

Introduction

Meat is the muscle fiber of an animal that is consumed as food. It is rich in water (75%), protein (20%), and fat (5%). Muscle fiber of an animal is also contained carbohydrates, vitamins, and minerals. In most regions of the world, the consumption of meat products continues to elevate [1]. Due to meat products are favored around the world, mislabeling of meat products have become common and can lead to economic deceit [2]. Usually, customers depend on the product labeling accuracy to make right choices for public health and religious causes [3].

Strategies have been utilised to identify meat species in products are based on either protein or DNA measurements. Protein detection for species of meat is unable to identify between species which are close relatives compare with DNA detection[4]. However, DNA detection for species of meat is a reliable, effective, simple and rapid method [5]. In previous studies, identification species of meat by DNA-based technique revealed the use of a label that is incorrect for sale under the name of another product. Since becoming aware of these issues, this work aimed to detect the meat species in products

Corresponding Author: Alaa Tariq Abdulwahid, Assistant Professor, Department of Veterinary Public Medicine, College of Veterinary Medicine, University of Basrah, Iraq.

E-mail: alaa.alsandaqchi@uobasrah.edu.iq

under laboratory conditions to prevent the sale of adulterated meat products.

Materials and Methods

Sample collection

Twenty seven canned meat products were collected from different commercial markets in Basrah province/ Iraq. Following collection, canned meat products were kept at room temperature until analysed. The Commercial canned meat products analysed for authentication are shown in Table 1.

Table 1: Commercial canned meat products analysed for adulation

Sample ID	Product label	Trademark	Source
1	Beef Luncheon Meat	Baidar	Kingdom of Saudi Arabia
2	Chicken Luncheon Meat	Baidar	Kingdom of Saudi Arabia
3	Beef Luncheon Meat	Hena	United Arab Emirates
4	Chicken Luncheon Meat	Hena	United Arab Emirates
5	Beef Hot Dog	AlTaghziah	Lebanon
6	Chicken Hot Dog	AlTaghziah	Lebanon
7	Beef Luncheon Meat	Ghadeer	Jordan
8	Corned Beef Loaf	Burdon	Brazil
9	Beef Hot Dog	Al Qaisar	Kingdom of Saudi Arabia

Preparation of sample for DNA extraction

Small pieces (2 gram) of sample were collected from each canned meat products using sterile forceps and mixed thoroughly with distal water (60 ml) in a blender (at 230 rpm for 120 sec) to homogenize. The sample was then put in microcentrifuge tube, labeled with the sample ID and stored to prevent DNA degradation at -20 °C.

DNA extraction and detection

DNA extraction was carried out in triplicate for all canned meat products using DNA extraction kit GsyncTMDNA (Geneaid Biotech Ltd., Taiwan). It was done as described by the manufacturer's instruction. Briefly, the meat product sample (25 mg) was lysed with ATL buffer (200 µL) and Proteinase K (20 µl), vortexed at 30 min, and incubated overnight at 60 °C. GSB buffer (200 µl) and absolute ethanol (200 µl) were then added to each tube and the tubes were revortexed for 10 sec. The samples were then put in GC columns, washed with W1 buffer (400 µl), centrifuged (14,000 xg for 30 sec), followed by second washed with W2 buffer (600 µl),centrifuged (16,000 xg for 30 sec), and discard the flow through. To dry the column matrix, the GC column centrifuged (16,000 xg for 3 min). After that, the dried column was put in a sterile microcentrifuge tube (1.5 ml) to add elution buffer(100 µl), stand at room temperature (3 min) to allow elution buffer, and centrifuged (16,000 xg) to elute purified DNA (30 sec). DNA qualities were then checked on agarose gel (1.5%). DNA quantities were identified by spectrophotometer (Nano Drop Technologies, Wilmington, USA).

PCR primers and amplification

The primers described in previous study[6] were used in the current study for PCR assay (Table 2).

Table 2: Primer sequence and target fragment for PCR assay

Species	primer sequence	Target fragment
Cattle	5'-TTAGTTGAATTAGGCCATGAAGCA-3' 5'-GTTTAAATAGGGTTAAGATGCACTCAATC-3'	84 bp
Sheep	5'-CTAAGAATAGAGTGCTTAGTTGAACCAGG-3' 5'- GTCTCCTCTCGTGTGGTTCAGATA-3'	121 bp
Goat	5'-AAACGTGTTAAAGCACTACATC-3' 5'-GTCTTAGCTATAGTGTATCAGCTGCA-3'	122 bp
Horse	5'-GACACACCCAGAAGTAAAGACA-3' 5'-TGCTGGGAAATATGATGATCAGA-3'	145 bp
Donkey	5'-TGCTAGCCTCATTATCAGTAT-3' 5'-GTGATGAGGATACGTGCT-3'	83 bp
Chicken	5'-TGAGAACTACGAGCACAAAC-3' 5'-GGGCTATTGAGCTCACTGTT-3'	183 bp

Polymerase Chain Reaction (PCR) assay for gene amplification

DNA from the collected samples was amplified a total 25 µL reaction volume containing genomic DNA (1µg) of each specie, primers (1µM), Mgcl2 (2mM), dNTP (0.2mM), PCR buffer (2.5µL of 10X) and the enzyme Taq DNA polymerase (1unit). The PCR assay were applied by thermal cycler as follows: initial denaturation step(94°C for 4 min) followed by 30 cycles of: denaturation step (94◦C for 30 sec), annealing step (57-64°C for 30 sec), extension step (72°C for 30 sec), and a final elongation (72◦C for 30 sec).

PCR products detection

The amplified PCR product was detected on agarose gels (1.5%) prepared with agarose in Tris-borate-EDTA buffer (1x) at 100 V for 30 min, stained with dye (Ethidium Bromide), and images using gel documentation systems (UVIdoc, UK). The size of the band was determined by comparison with a standard DNA ladder (New England Biolabs).

Results and Discussion

Mitochondrial DNA fragments of cattle (84pb), sheep (121pb), goat (122pb), horse (145pb), donkey (83pb), and chicken (183bp) were amplified. PCR analysis of canned meat revealed negativity results of all samples for regarding to sheep, goat, horse, and donkey meats (Table 3, Figure 1-4). However, the current study revealed that chicken and beef meat were found in the canned meat products that are not declared in their labels (Table 3, Figure 5-6).

Adulation by exchange or mixing undeclared species in meat products under food labeling regulations is illegal. Detecting the meat species used in a meat product is an importance issue in food industry. The current study revealed that there was mislabeling in analysed samples. This finding is in agreement with previous studies, in which mislabeling was reported in different marketed meat products. In Turkey, meat products that were declared as beef contained poultry and donkey meat [6]. In addition, raw meat that was declared as beef contained mix of horse and deer meat [7]. In china, meat products that were declared as buffalo contained cattle, pork, and duck meat [5]. In Iran, raw burgers that were declared as beef contained poultry meat [8]. In France, 18% of ground meat samples collected from local European and Asian markets revealed adulteration cases with undeclared meat species [9]. In Bangladeshi, up to one third of beef-labeled products revealed adulteration cases of products with buffalo and chicken [10].

In the present study, chicken meat was detected undeclared animal species. The main reason for the exchange of chicken flesh (cheaper) in beef (more expensive) is economic. Another reason might be an accidental cross contamination can occur

during handling, the use of shared equipment, and processing [11]. The meat species exchange occurs usually in processed meats (canned meat, salami, sausages, and sucuk). The main reason for success the adulteration in the processed meats is processing technique. This technique causes invisible changes in the physical appearance (color, texture, and flavor). So, detection of adulation by visual inspection is more difficult in processed meats than in fresh meat[10].

The current study and the others found that all countries have requirements about authenticity and labeling. In general, chicken meat exchange is common in Muslim countries because of cheaper than beef meat.

Table 3: PCR results for canned meat product

Product label		Adulteration ingredients PCR results					
		Cattle	Sheep	Goat	Horse	Donkey	Chicken
1	Beef Luncheon Meat/Baidar	+	–	–	–	–	+
2	Chicken Luncheon Meat/Baidar	+	–	–	–	–	+
3	Beef Luncheon Meat/Hena	+	–	–	–	–	+
4	Chicken Luncheon Meat/Hena	+	–	–	–	–	+
5	Beef Hot Dog/AlTaghziah	+	–	–	–	–	+
6	Chicken Hot Dog/AlTaghziah	+	–	–	–	–	+
7	Beef Luncheon Meat/ Ghadeer	+	–	–	–	–	+
8	Corned Beef Loaf/Burdon	+	–	–	–	–	+
9	Beef Hot Dog/Al Qaisar	+	–	–	–	–	+

Note: (+) denotes for presence and (-) stands absence

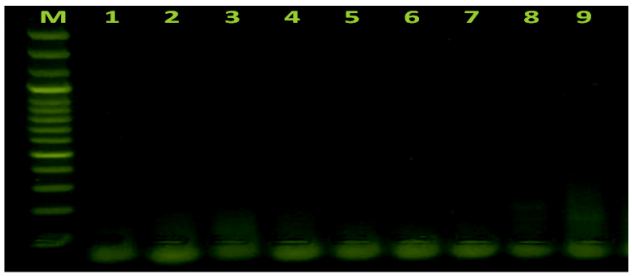

Figure 1: PCR product with 121pb using sheep specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to sheep meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/ Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/ AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.

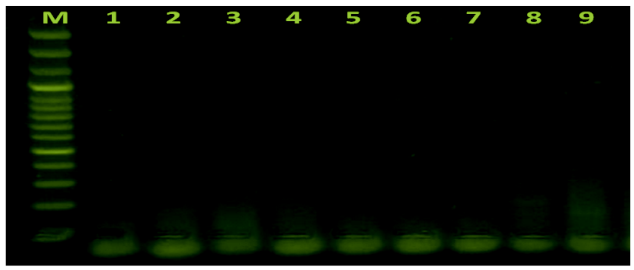

Figure 2: PCR product with 122pb using goat specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to goat meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/ Burdon; lane 9: Beef Hot Dog/Al Qaisar.

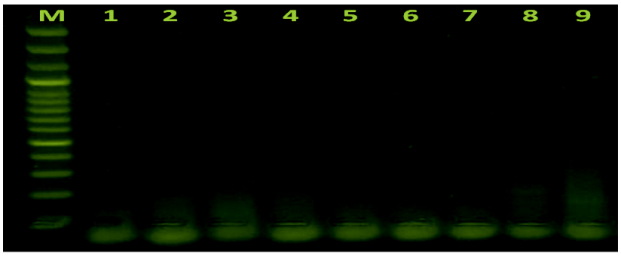

Figure 3: PCR product with 145pb using horse specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to horse meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/ Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/ AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.

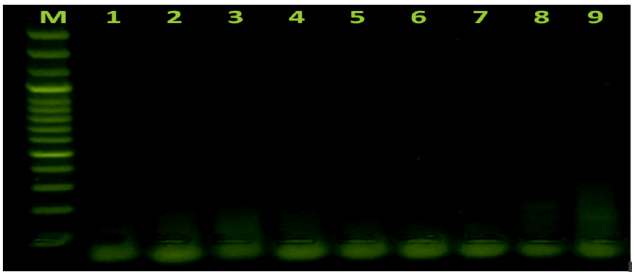

Figure 4: PCR product with 83pb using donkey specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negatively result of all samples for regarding to donkey meat (L1- L9) M: molecular marker; lane 1: Beef Luncheon Meat/ Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/ AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.

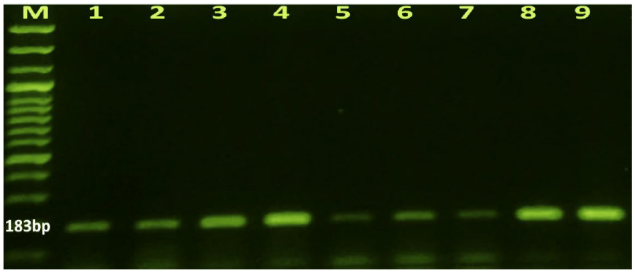

Figure 5: PCR product with 183bp using chicken specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the positively result of all samples for regarding to chicken meat (L1- L9). M: molecular marker; lane 1: Beef Luncheon Meat/ Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/ AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/Burdon; lane 9: Beef Hot Dog/Al Qaisar.

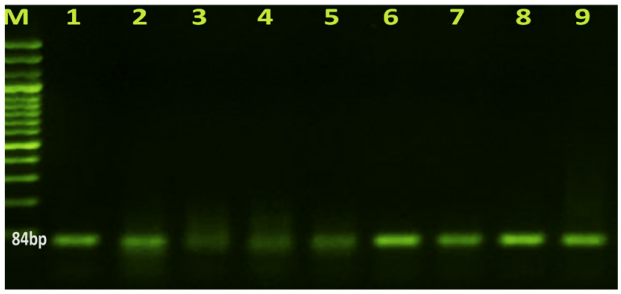

Figure 6: PCR product with 84bp using cattle specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the positively result of all samples for regarding to cattle meat (L1- L9). M: molecular marker; lane 1: Beef Luncheon Meat/Baidar; lane 2: Chicken Luncheon Meat/Baidar; lane 3: Beef Luncheon Meat/Hena; lane 4: Chicken Luncheon Meat/Hena; lane 5:Beef Hot Dog/AlTaghziah; lane 6: Chicken Hot Dog/AlTaghziah; lane 7: Beef Luncheon Meat/ Ghadeer; lane 8: Corned Beef Loaf/ Burdon; lane 9: Beef Hot Dog/Al Qaisar.

Conclusion, mislabeling of canned meat with cheaper protein is a problem. To protect the consumer to make right choices when purchasing canned meat, canned meat must be regularly analysed using effective methods.

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Ethical Clearance: Taken from the scientific Committee, University of Basrah.

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