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ORIGINAL ARTICLE

Molecular Studies to Identify to Commercial Fraud in Processed Meat in the Egyptian Market

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ABSTRACT

Background*: The rising consumption of meat products such as luncheon meat and kofta in large urban areas has underscored the importance of quality control and accurate labeling. With the increasing cost of red meat and the difficulty of detecting adulteration in ground beef, it is likely that cheaper animal and plant proteins are being substituted for beef in these products.* ***Objective:*** *This study aimed to evaluate the accuracy of labeling in beef luncheon and premium kofta products. A sensitive and rapid uniplex-PCR technique was employed to detect the presence of pig, mouse, dog, cat, and chicken meats-species considered indicators of commercial fraud in such products.* ***Methodology:*** *Samples were collected from markets in Shebin El Kom City, Egypt, including five brands of premium kofta and five brands of luncheon meat made with beef. Species-specific primers were used to optimize uniplex-PCR for beef, chicken, pig, mouse, cat, and dog meat detection. Mitochondrial genes and RNA were targeted for the specific and qualitative identification of cat, dog, and rodent (mouse or rat) tissues in food.* ***Results****: the results revealed the presence of undeclared meats-including pig, cat, chicken, mouse, and dog—in the tested kofta and luncheon products. Although 5% of the samples were free from pig residues, the majority were contaminated with unlisted animal meats. Notably, one brand of luncheon meat contained no beef at all.* ***Conclusion:*** *The uniplex-PCR assay is a rapid and dependable diagnostic tool that can be routinely employed by quality control laboratories to detect meat fraud and ensure food authenticity.*

INTRODUCTION

Meat is considered one of the most nutritious and healthiest sources of protein. However, due to its high cost and the intense competition among producers for profit, adulteration in meat products has become widespread[1]. This includes the substitution of plant or animal proteins, use of inferior raw materials, incorporation of meat from species with lower commercial value, use of unidentified species, inaccurate ingredient reporting, or complete omission of certain ingredients[2,3,4,5]. Moreover, preventing food fraud and protecting consumer rights has become increasingly important, as modern consumers are more concerned with various factors such as diet (e.g., calorie and nutrient content), religion (e.g., avoidance of pork), lifestyle (e.g., preference for organic and vegetarian foods), and health issues (e.g., the presence of allergenic compounds)[1]. Given these concerns, the evaluation and validation of the basic ingredients in meat products has become a primary focus for professionals in the food industry[6]. On one hand, inaccurate food labeling represents a form of adulteration that particularly worries consumers, as it often involves substituting a high-value animal species with a cheaper alternative. On the other hand, incorrect labeling may also fail to disclose allergens, posing significant risks to consumers who are allergic or sensitive to those ingredients[2].

The European Commission Law 178/2002 defines validation as the ability to identify animal species and products at various stages of the food production chain, from production through to distribution[4]. To ensure the quality and safety of food products for consumers, rapid verification of their authenticity and reliability is essential. Increasing global demands to determine the origins of food products, assess health risks associated with consuming adulterated food, and understand the environmental and human food chain effects of genetically modified organisms have made this requirement even more critical[5].

If a product contains a combination of chicken and red meat, the meat with the highest proportion must be identified on the raw material label. All ingredients used in the production of meat and poultry products must be declared in compliance with the Appendix criteria. Additionally, products containing red meat, chicken, and plant proteins must indicate the name of the plant protein on the ingredients label alongside the product name[7] if the ratio of plant protein to animal protein is less than 1:13 or equal to 1:10.

Processed meat products are those that combine meat with fat, water, and other non-meat ingredients. These products are rich in essential nutrients, including vital amino acids, minerals, and trace elements[8]. Due to its high biological value, affordability, palatable taste, and ease of preparation, Egyptian beef luncheon is considered one of the most popular ready-to-eat meat products. However, overconsumption of processed meats has been linked to serious health conditions such as heart disease, hyperglycemia, tumors, and liver, kidney, and lung disorders[9].

In Egypt, cooked beef luncheon is viewed as a traditional meat product widely consumed by the public. It is made from finely chopped pork that is cured with salt and nitrite and then heat-treated[10]. The cost of luncheon meat is determined by the amount of lean meat it contains and the quality of its raw materials[11]. Nevertheless, to reduce production expenses, some manufacturers resort to using low-cost, low-quality ingredients[12]. Compared to non-consumers, those who frequently consume beef luncheon tend to have higher intakes of calories, protein, trace elements, and fat[13].

Understanding the composition of meat products significantly affects their shelf life, pricing, functional properties, sensory characteristics, nutritional value, and quality control[14],[15]. Due to the varying cuts of beef, use of food additives, and different preparation techniques (such as curing, salting, drying, and heating), the nutritional content and sensory properties of processed meats can differ greatly from product to product[16].

Kofta, also known as meatballs, is another traditional meat-based product. It is made from minced beef mixed with refined wheat flour or Bengal gram flour and 10–20% hydrogenated fat to create an emulsion. Spices and condiments are then added in precise amounts to enhance flavor, and the mixture is carefully shaped into spherical forms.

Microbial and chemical analyses are used to ensure the quality of these products according to national standards. However, one of the major challenges with luncheon and kofta products is the adulteration carried out by unscrupulous manufacturers who incorporate cheap, unauthorized ingredients, thereby exposing consumers to substandard goods[17]. Unlike bone-in or whole cuts, identifying the species origin of meat in processed products is more difficult due to structural changes resulting from extensive industrial processing. This makes it possible to introduce and conceal various protein sources[18],[19].

One common form of adulteration involves the partial substitution of beef with chicken meat. Given that chicken has lower levels of saturated fat and cholesterol than red meat, there is a high likelihood that manufacturers mix mechanically deboned chicken with minced red meat[20]

In recent years, numerous techniques have been developed to detect the various animal and plant protein species present in meat products. These methods include chemical analysis, sensory evaluation, histological observation, fat tissue examination, muscle glycogen measurement, electrophoresis, immunological assays, chromatography, and DNA hybridization. However, each of these approaches has limitations, such as lack of specificity, high cost, and time-consuming protocols[21].

DNA-based techniques have emerged as promising tools for identifying species in animal products due to DNA's inherent stability, its species-specific sequences, and its resistance to industrial processing conditions such as high temperatures, pressure, and chemical treatments[22]. Among these techniques, polymerase chain reaction (PCR) is considered the most advanced molecular tool for identifying meat species, added non-meat proteins, and allergens in complex food mixtures. This is due to its simplicity, rapidity, specificity, sensitivity, reproducibility, and low detection threshold[23,24,25,26].

DNA-based methods such as real-time PCR, multiplex-PCR, restriction fragment length polymorphism (RFLP), species-specific PCR, random amplified polymorphic DNA (RAPD), and single-strand conformation polymorphism (SSCP) have been widely utilized[27,28]. In this study, a sensitive and specific uniplex-PCR approach was employed using species-specific primers for detection[22]. Uniplex-PCR was chosen because it allows for the simultaneous detection of multiple target DNAs in a single reaction phase, reduces the number of required reactions, saves time and cost, and offers excellent detection sensitivity in a single-stage PCR reaction involving mixed DNA samples from different species[29,30,31].

The aim of the work is to detect and identify tissues of cats, mice, dogs, cattle (Bos), chickens, and pigs in luncheon and kofta meat products by applying a uniplex-PCR method targeting the 12S ribosomal RNA mitochondrial gene.

METHODOLOGY

Sample collection and processing

In the Menoufia Governorate of Egypt, thirty fresh muscle samples (five samples from each meat species) were obtained from local butchers. This included beef (Bos) and chicken (Poultry). Since it was not possible to obtain dog or cat meat, five hair samples from five different cats and five hair samples from five different dogs were collected as alternative sources of dog or cat DNA. Additionally, a small mouse was dissected, and its vital organs, including the liver, spleen, kidney, and heart, were removed. Lastly, a pork sample was purchased from a slaughterhouse in Mukattam, intended for use by Christians.

Luncheon and kofta samples from several commercial brands were collected from the market to serve as the basis for the experiment. Five distinct

brands of kofta and five different types of luncheon meat were gathered. In addition to the reference samples, these samples were refrigerated until the practical trials began. To prevent cross-contamination, each meat sample was placed in a zip-lock plastic bag, labeled with a unique identifier, and kept separately. The samples were stored in the refrigerator at 4°C until further processing, and then preserved in 100% alcohol

Oligonucleotid primers

Different parts of the mitochondrial genome were amplified using species-specific primers, targeting the same gene12S ribosomal RNA mitochondrial gene for species (table 1)

Table 1: The target species, primer sequences, and amplicon sizes are shown below, along with the sequence of primers and expected product size:

Primers Species		Sequences	Amplicon bp
Poultry / Chicken	F	TGAGAACTACGAGCACAAAC	183bp
	R	GGGCTATTGAGCTCACTGTT	
Cat	F	AATTGAATCGGGCCATGAA	108bp
	R	CGACTTATCTCCTCTTGTGGGTGT	
ruminant	F	GCCATATACTCTCCTTGGTGACA	271bp
	R	GTAGGCTTGGGAATAGTACGA	
Dog	F	AATTGAATCGGGCCATGAA	101bp
	R	CTCCTCTTGTGTTTTAGTTAAGTTAATCTG	
Pig	F	AACCCTATGTACGTCGTGCAT	531bp
	R	ACCATTGACTGAATAGCACCT	
Mouse	F	AAATCCAACTTATATGTGAAAATTCATTGT	96 bp
	R	TGGGTCTTAGCTATCGTCGATCAT	

Isolation of genomic DNA

DNA was extracted from specific animal tissues using a modified "salting out" extraction method. A lysing solution containing 5 M NaCl was used to precipitate the proteins. For tissue lysis, 50 mM NaCl, 1 mM Na2EDTA, 0.5% SDS, and pH 8.3 buffer were used to lyse 10 milligrams (0.01 g) of tissue in Eppendorf tubes, and the samples were gently shaken. After shaking, 200 microliters of saturated NaCl were added, and the mixture was incubated overnight at 37°C. Following incubation, the mixture was centrifuged for 10 minutes at 12,000 rpm. The supernatant was then transferred to new Eppendorf tubes, and 600 microliters of cold isopropanol were added to precipitate the DNA. The mixture was inverted several times until fine fibers began to appear, and then centrifuged for 5 minutes at 12,000 rpm. After centrifugation, the alcohol was decanted, and the tubes were dried by blotting with Whatman paper. The DNA pellets were then resuspended in 50 microliters (or an appropriate volume) of TE buffer (10 mM Tris, 1 mM EDTA, PH 8) containing 5% glycerol. Incubating the resuspended DNA with a loading mix (RNase + loading buffer) for 30 to 60 minutes.

Polymerase chain reaction (PCR)

PCR amplification was performed using both universal and species-specific primers. A total of universal primers were used to amplify a partial segment of the 12S ribosomal RNA mitochondrial gene [32,33,34,35]. The amplification was carried out with a 25 µL master mix comprising 2 µL of DNA, 0.4 µM of each primer, and IX MyTaq™™ Red Mix (Bioline, Cat. No. BIO-25043).

For the amplification reactions, the following components were used in a 25 µL total volume: 1 µL of template DNA (50 ng), 0.5 µM of each primer (1 µL), 12.5 µL. of IX MyTaq™™ Red Mix, and 1.25 µL of bovine serum albumin (BSA, 4 mg/mL). PCR-grade water was added to complete the reaction volume to 25 µL. The PCR program on the Biometra T-Personal (Germany) heat cycler was as shown in table 2.

Table 2: PCR programs for samp12 PCR of ruminant, poultry, pig, cat, dog and mouse DNA

Species	Preheating	Denaturation	Annealing	Extension	Final Extension
Ruminant	95^{0}c for 5 minute	95^{0}c for 1minute	63^{0}c for 1 minute	72^{0}c for 1 minute	72^{0}c for 6 minute
Poultry	95^{0}c for 5 minute	95^{0}c for 1 minute	60^{0}c for 1 minute	72^{0}c for 1 minute	72^{0}c for 6 minute
Pig	95^{0}c for 5 minute	95^{0}c for 1 minute	61^{0}c for 1 minute	72^{0}c for 1 minute	72^{0}c for 6 minute
Cat \Dog	93^{0}c for 2 minute	93^{0}c for 30 second	60^0 for 30 second	72^{0}c for45 second	72 ^{0}c for 3 minute
mouse	93^{0}c for 2 minute	93^{0}c for 30 second	55^{0}c for 30 second	72^{0}c for 45 second	72 ^{0}c for 3 minute

Agarose gel electrophoresis

After completing the PCR process, the samples were subjected to electrophoresis using a 2% agarose gel. The agarose gel was prepared by adding 0.6 g of agarose to 30 ml. of IX TBE buffer. The gel was then stained with 0.5 µg/mL of ethidium bromide and poured into a 12-well comb gel tray. Eight µL of each PCR product and a 1 kb DNA ladder were loaded into the wells. The electrophoresis was run at 75 volts for 25 minutes. The PCR bands were visualized using a UV transilluminator.

RESULTS

The extracted DNA from the control samples—beef, chicken, cat, dog, pig, and mouse—was first subjected to uniplex-PCR using species-specific primers. The primers targeted the 12S mitochondrial ribosomal RNA gene. To verify the distinct function of every primer and prevent primer cross-reaction, uniplex-PCR was performed three times on non-target DNA for each primer, confirming its specificity (figure 1). Figures 2-7 show the uniplex-PCR amplification findings on a 2% agarose gel for 10 brands of Lancheon and Kofta, using species-specific primers for beef, poultry, cat, dog, pig, and mouse. The results revealed that all lancheon and kofta samples contained beef, chicken, cats, dogs, pigs, and mice, except for one brand of lancheon (lane 8, brand cc) which did not contain beef. Additionally, lane 1,2,7,10 did not contain pig meat.

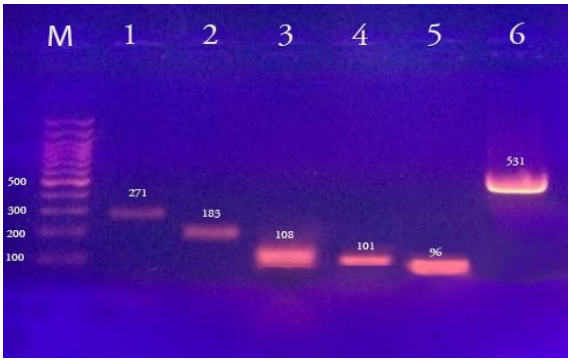

Fig. 1: 2% Agarose gel electrophoresis of stander uniplex PCR assay of all six species.
Lane M: 100 bp DNA ladder (molecular marker); lane 1: beef (271 bp); lane 2: poultry (183 bp); lane 3: Mouse (96 bp); lane 4:Cat (108 bp) ; lane5:Dog(101 bp);lane 6: pig (531 bp); and lane 7 anther lane of beef .

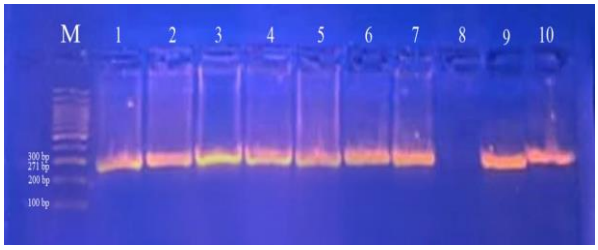

Fig. 2: Uniplex PCR results of lancheon and kofta samples targeting boss gene; M 100 bp DNA ladder (Gene Ruler).
[Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)]

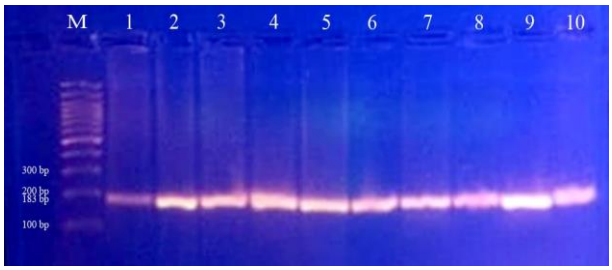

Fig. 3: Uniplex PCR results of lancheon and kofta samples targeting chicken gene; M 100 bp DNA ladder (Gene Ruler)
[Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)]

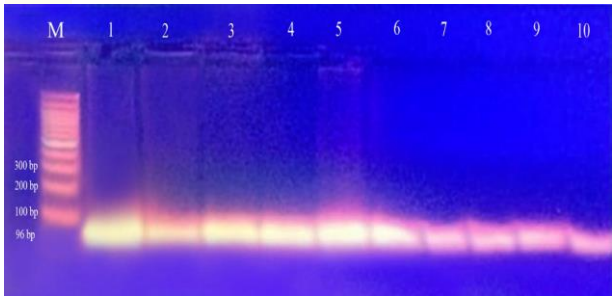

Fig. 4: uniplex PCR results of lancheon and kofta samples targeting mouse gene; M 100 bp DNA ladder (Gene Ruler)
Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)]

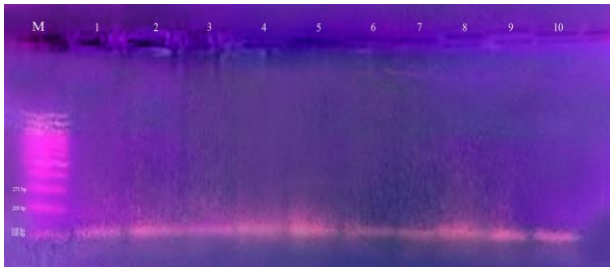

Fig. 5: Uniplex PCR results of lancheon and kofta samples targeting cat gene; M 100 bp DNA ladder (Gene Ruler)
[Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)]

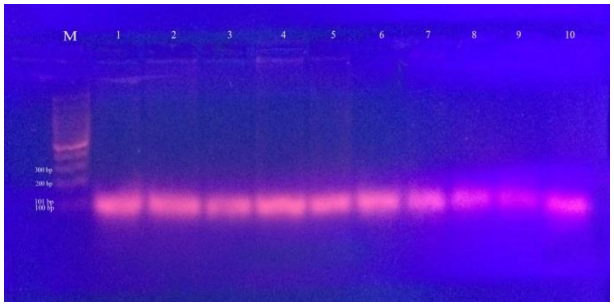

Fig. 6: Uniplex PCR results of lancheon and kofta samples targeting dog gene; M 100 bp DNA ladder (Gene Ruler)
[Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)

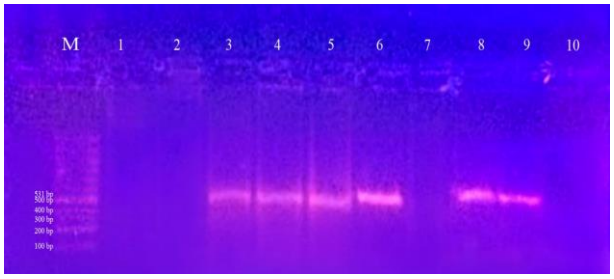

Fig. 7: Uniplex PCR results of lancheon and kofta samples targeting pig gene; M 100 bp DNA ladder (Gene Ruler)
[Lancheon Samples (1:5) lane1 (brand A); lane2 (brand B); Lane3 (brand C); lane4 (brand D); lane5 (brand E)]
[kofta Samples (6:10) lane6(brand aa); lane7(bb); lane8(cc); lane9(dd); lane10(ee)]

DISCUSSION

Extracting the relevant DNA was the first step in the PCR test. The extracted DNA from control samples of beef, chicken, cat, dog, pig, and mouse was separated and rendered visible using agarose gel 2% electrophoresis in order to evaluate the DNA's quality. The amount and purity of the isolated DNA are crucial in addition to its quality[22]. Quantitative studies revealed that the control and lancheon and kofta DNA had purity values between 1.8 and 2, indicating a high amount of extracted DNA, high extraction process efficiency, and compatibility for PCR amplification[20,36].

The universal meat adulteration problem produced a series of grave inconveniences, such as infringing the labeling laws, economic fraud, and conflicts with religious, ethical, and hygienic concerns[35]. Hence, the development of fast and accurate routine methods should be carried out for quality control to assure public food safety all over the world. Species discrimination in case of processed meats is difficult, since heating, smoking, and mechanical processing severely change tissue texture and, hence, degrades meat DNA[36]. Yet, still the short DNA fragments left after such treatments are recoverable enough for DNA fingerprinting and molecular identification[37]. As they have many advantages over other analyses, DNA tests are now widely used techniques in practice. Also, in this case as previously described here in, DNA is found in all tissues and is more stable than genomic DNA [37,38]. Such identification can contribute much to the crucial requirements for assuring food safety, complying with consumer demands and respecting laws. Clearly as of right now, the PCR assay is the method of choice for identifying meat species via DNA analysis. Recently, PCR and its derivative meat authentication methods—such as species-specific PCR, DNA barcoding, multiplex PCR, PCR-RFLP, and QPCR—have been employed[39,40]. Meat authenticity research of commercial meat products in Egypt is still modern.

CONCLUSION

We can conclude that the uniplex-PCR assays used in this study demonstrated sufficient sensitivity to detect meat species within complex mixtures. DNA fingerprinting, as demonstrated in this study, proved to be a highly accurate method for identifying species under various environmental, mechanical, and chemical conditions.

Conflicts of Interest
The authors declare that there are no conflicts of interest.
Consent to participate: Informed consent was obtained from all individual participants included in the study.
Consent to publish: Informed consent was obtained to publish the data.

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